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(54) 25-HYDROXYVITAMIN D3 24-HYDROXY ENZYME GENE-TRANSDUCED ANIMAL

(57)Abstract:
PROBLEM TO BE SOLVED: To obtain the subject animal most suitable for utilization thereof aimed at elucidation, etc., of the function of a vitamin D3 24-hydroxy enzyme by allowing the animal to have a DNA with incorporated exogenous 25- hydroxyvitamine D3 24-hydroxy enzyme gene, or a mutative gene thereof.

SOLUTION: The objective nonhuman mammal is obtained by allowing an animal to have a DNA with incorporated exogenous 25-hydroxyvitamine D3 24-hydroxy enzyme gene, or a mutative gene thereof. The nonhuman mammal can be a rabbit, a dog, a cat, a guinea pig, a hamster, a mouse or a rat and is preferably the rat. A material used for preventing and treating a disease caused by vitamin D3 metabolic disorder can be screened by applying a material to be tested to the animal or a part of the creature, and assaying the improving effect of the disease caused by the vitamin D3 metabolic disorder by the material to be tested.

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CLAIMS

[Claim(s)]
[Claim 1] A part of nonhuman mammal which has DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle, or its living body
[Claim 2] A part of animal according to claim 1 whose nonhuman mammal is a rabbit, a dog, a cat, a guinea pig, a hamster, a mouse, or a rat, or its living body
[Claim 3] A part of animal according to claim 1 whose nonhuman mammal is a rat, or its living body
[Claim 4] The vector which contains a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle, and may discover this gene in mammalian.
[Claim 5] The screening approach of the matter used for prevention and the therapy of the disease resulting from a vitamin-D3 metabolic error which applies an examined substance to a part of nonhuman mammal which has DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle, or its living body, and is characterized by authorizing the improvement effect of the disease resulting from the vitamin-D3 metabolic error of this examined substance.
[Claim 6] The screening approach according to claim 5 that the disease resulting from a vitamin-D3 metabolic error is kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, or cancer.
[Claim 7] The screening approach according to claim 5 that the disease resulting from a vitamin-D3 metabolic error is kidney disease or a bone disease.
[Claim 8] The remedy for prevention / therapy of the disease resulting from

the vitamin-D3 metabolic error which comes to contain the matter judge that has the improvement effect of the disease which originates in a vitamin-D3 metabolic error by the approach according to claim 5.
[Claim 9] The remedy according to claim 8 whose disease resulting from a vitamin-D3 metabolic error is kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, or cancer.
[Claim 10] The remedy according to claim 8 whose disease resulting from a vitamin-D3 metabolic error is kidney disease or a bone disease.
[Claim 11] The application for screening the matter used for prevention and the therapy of the disease resulting from some vitamin-D3 metabolic errors of the nonhuman mammal which has DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle, or its living body.
[Claim 12] Some production approaches of the rat according to claim 3 characterized by introducing DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle into the fertilized egg which is made to cross the female rat which prescribed luteinizing hormone 0 [about] thru/or 10IU / individual for the patient with a male rat, and is obtained after prescribing follicle-stimulating hormone 20 [about] thru/or 50IU / individual for the patient, and implanting this fertilized egg to a female rat, or its living body.
[Claim 13] Some production approaches of the rat according to claim 3 characterized by implanting the fertilized egg which introduced DNA which included a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle in the male rat and the pseudopregnancy rat of the female made to cross after prescribing luteinizing hormone releasing hormone or its analog for the patient, or its living body.

[Translation done.]

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to a 25-hydroxylation vitamin D 324-hydroxylase transgenic animal.
[0002]
[Description of the Prior Art] A transgenic animal has the gene introduced in the initial (usually single cell) developmental stage into the animal or the germ line of the ancestor of this animal. Stewart (1981, proceedings OBU National academy OBU Science (ProNAS, USA), the 78th volume, the 5016th page) (Stewart), such as Wagner (Wagner), etc. has indicated the trans-genic mice containing a Homo sapiens globin gene (1982, Science (Science), the 217th volume, the 1046th page). RESHI (1981, Nature (Nature), the 294th volume, the 92nd page) (Lacy), such as KONSUTANCHINI (Constantini), etc. has indicated the trans-genic mice containing a rabbit globin gene (1983, a cel (Cell), the 34th volume, the 343rd page). The Mac night (McKnight) etc. has indicated the trans-genic mice containing a transferrin gene (1983, a cel, the 34th volume, the 335th page). The BURIN star (Brinstar) etc. has indicated the trans-genic mice containing the immunoglobulin gene introduced functionally (1983, Nature, the 306th volume, the 332nd page). The main trans-genic mice which participate in a bone disease have the next report. Lewis (Lewis, D.B.) etc. had indicated mouse lck-IL4 trans-genic mice (1993, proceedings OBU National academy OBUSAIENSU, the 90th volume, the 11618th page), and in this mouse, there were few amounts of osteocalcin intentionally, and it reported that the osteoporosis's symptom was seen.
[0003] On the other hand, the main trans-genic mice which participate in kidney disease have the next report. It reported Doi (Doi, T.) etc. having indicated bovine growth hormone trans-genic mice (1988, American journal OBU PASOROJI (Am.J.Pathol.), the 131st volume, the 398th page), and glomerulosclerosis having advanced by seven-week ** in this mouse above

diffusible mesangium sclerosis and 30-week **, and having died of uremia. DORESURA (Dressler, G.R.) etc. had indicated Pax-2 trans-genic mice (1993, Nature (Nature), the 362nd volume, the 65th page), the gene expression in the kidney was checked in this mouse, and it reported that a symptom similar to nephrosis, such as glomerulus withering and albuminuria, was seen. Moreover, Loden (Lowden, D.A.) etc. had indicated TGF-alpha trans-genic mice (1993, journal OBU laboratory clinical Mehdi Soon (J.Lab.Clin.Med.), the 124th volume, the 386th page), the gene expression in the kidney was checked in this mouse, and it reported that the symptom of the nephrocystosis and glomerulus hypertrophy was seen. However, the foreignness transgenics rat which can serve as a model of a bone disease or kidney disease is not known now.

[0004] Although two types, D2 and D3, exist in a nature, and D2 has a double bond in the 22nd place of a side chain, they have a methyl group in the 24th place and vitamin D exists in vegetation, it is known that D3 exists in an animal. With 7-hydronalium cholesterol (provitamin D 3) generated on the human skin, the optical cleavage reaction for which the 9th place of B ring and the 10th place cleave occurs, and previtamin D 3 is generated by the ultraviolet rays of 290 to 320 nm UV. Furthermore, it isomerizes according to temperature and generates vitamin D3. This vitamin D3 shows the structure where B ring cleaves and three double bonds are located in a line every other one. Vitamin D3 combines with vitamin D joint protein, and is conveyed to liver. 25-hydroxylase which exists in the mitochondrion of hepatocyte hydroxylates the 25th place of a side chain. It becomes 25-hydroxylation vitamin D3, and it is continuously conveyed to the kidney, and are dependent on the calcium metabolism regulatory hormones in blood and calcium concentration, such as PTH. With a proximal tubule 1 alpha position, The 23rd place and 24 It hydroxylates grade and the 26th place, respectively, and becomes 1alpha, 25-hydroxylation vitamin D3, 23, 25-hydroxylation vitamin D3, 24, 25-hydroxylation vitamin D3, 26, and 25-hydroxylation vitamin D3. Among those, the high things of bioactive are 1alpha and 25-hydroxylation vitamin D3, and bone metabolism, such as raising the calcium in blood and the Lynn concentration, and acceleration and production of proteoglycan raising [the manifestation of osteopontin and osteocalcin] production of cell membrane production phospholipid control and reversely to osteoblast, consequently promoting mineralization of osteoblast, occurs. As a function of 24 and 25-hydroxylation vitamin D3, formation of an osteoclast is controlled, promoting osteocalcin gene expression is known, and although promoting mineralization in a vitamin D deficiency rat is also reported, the operation is based on the function of the 25-hydroxylation vitamin D 324-hydroxylase (called vitamin D 324-hydroxylase or 1alpha, and 25-hydroxylation vitamin-D3 hydroxylase) which exists in a proximal tubule. Moreover, it is also known for

the latest research that this enzyme hydroxylates the 26th place also to except the 24th place. A vitamin-D-dependency II mold, rickets, osteomalacia, etc. are one of those which are known as an important disease of a vitamin D metabolic turnover. These diseases will become osteomalacia, if the condition of deformation of the osteogenesis imperfecta by the mineralization failure and a bone is shown, and a mineralization failure arises before metaphysis closeout, and it will become rickets and will be generated after closeout. In addition, it may become the kidney disease which includes renal failure independently as complication with the above-mentioned disease, secondary hyperparathyroidism, and a hypercalcemia.

[0005] Daisen (Ohyama) etc. (1989, FEBUSU Letters (FEBS Lett.), the 255th volume, the 405th page) Are made from the rat kidney mitochondrion which prescribed for the patient and extracted vitamin D. Purification of vitamin D 324-hydroxylase is succeeded and followed. Daisen and others (Ohyama) etc. (1991, FEBUSU Letters (FEBS Lett.), the 278th volume, the 195th page) cDNA was isolated by screening of the clone by the vitamin D 324-hydroxylase antibody. Vitamin D 324-hydroxylase cDNA has an overall-length 3.2K base and the reading frame (generally referred to as open reading frame) of 1542bp(s) which translate 514 amino acid among those, and generating the protein of molecular weight 59,000 is known. It became clear to become molecular weight about 55,000 protein which 35 amino acid is cut from the amino terminal of the generated protein, and consists of 479 amino acid and which matured. Since the 462nd [further] amino acid cysteine combined with the 5th place of a hem, it also becoming clear having the description of the mitochondrion mold protein P-450, and discovering it as protein by manifestation experiment in a COS cell was checked. On the other hand, Homo sapiens vitamin D 324-hydroxylase cDNA It is obtained by the chain (Chen) etc. (1993, proceedings OBU National academy OBU Science, the 90th volume, the 4543rd page). It of a mouse Ito (Itoh) (1995, biotechnology KEMIKA E biotechnology FIJIKA AKUTA (Biochemica et BiophysicaActa)) etc. It was obtained by the 1264th volume and the 26th page and it of a guinea pig was obtained by Daisen (Ohyama) etc. (1996, the Japanese Society for Bone and Mineral Research journal, the 14th volume, the 112nd page). It is clear that the amino acid sequence of a rat, Homo sapiens, a mouse, and this enzyme of a guinea pig has about 80 to 95% of homology mutually.

[0006] Analysis was performed also about the function in the living body, and Araki (Shinki) etc. made it clear from Northern analysis that vitamin D 324-hydroxylase was guided by 1alpha and 25-hydroxylation vitamin D3 (1992, journal OBU biological chemistry (J.Biol.Chem.), the 267th volume, the 13757th page). Moreover, it became clear that early and its extent also have [1-in that case alpha and 25-hydroxylation vitamin D3] a reaction higher than

the kidney in a small intestine. As a result of investigating the reactivity of 25-hydroxylation vitamin D3 and 1alpha, and 25-hydroxylation vitamin D3 to a pan, it was reasoned from the difference of Km value that the direction of 1alpha and 25-hydroxylation vitamin D3 had the high substrate specificity of vitamin D 324-hydroxylase. Moreover, it investigates about 25-hydroxylation vitamin-D3 metabolic turnover in the experiment by the Spodoptera frugiperda (Sf2l.) cell (1996, biochemistry (Biochemistry), the 35th volume, the 8465th page), and BEKKEN (Becken, M.J.) etc. is the 23rd place and 24. It suggested that both the catalyses of an about occurred. Furthermore, although vitamin D 325-hydroxylase is isolated from a rat liver mitochondrion by **** (Masumoto) etc. (1988, journal OBU biological chemistry (J.Biol.Chem.), the 263rd volume, the 14256th page) and the knowledge about this gene function is also acquired, now, neither isolation purification of vitamin D 31alpha-hydroxylase nor gene cloning has resulted in the success. The gene expression of vitamin D 324-hydroxylase has received accommodation, when the heterodimer of the vitamin-D3 acceptor (generally abbreviated to VDR) and retinoid X acceptor (generally abbreviated to RXR) which 1alpha and 25 hydroxylation vitamin D3 combined with vitamin-D3 response array (generally abbreviated to VDRE) VDRE-1 which exists in -150--136 of 5'upstream region joins together. This vitamin-D3 response array consists of repeat structure (generally tandem repeat ********) which sandwiched the gap of three bases of the motif which consists of six bases of AGGTCA, and similarity with the response array (generally abbreviated to TRE) of thyroid hormone and the response array (generally abbreviated to RARE) of a retinoic acid is pointed out to others. Furthermore, a kelly (Kerry, D.M.) etc. is set in the gene concerned (1996, journal OBU biological chemistry (J.Biol.Chem.), the 271st volume, the 29715th page). VDRE-1 which exists in -150--136 among three vitamin-D3 response arrays which exist in 5'upstream region - Rather than VDRE-2 which exist in 249--232, to 1alpha and 25-hydroxylation vitamin D3, it was high, and according to both collaboration operation, susceptibility is raising the activity and suggested adjusting the reaction to 1alpha and 25-hydroxylation vitamin D3. As a main gene which has received expression control precipitative by 1alpha and 25-hydroxylation vitamin D3 Alkaline phosphatase, the aldolase subunit B, a GURISERUARUDE hide-3-phosphoric-acid dehydrogenase, The heat shock protein -70, cull bottle gin-D 28K and 9K, Osteocalcin, osteopontin, osteonectin, fibronectin, Interleukin I-6, Matrix-gla-protein, metallothionein, The NADH-DH subunits III and IV, integrin alphaVbeta3, transformer foaming Growth Factor beta A nerve growth factor, c-FMS, c-fos, c-KI-RAS, A vitamin-D3 receptor, 25-hydroxylation vitamin D 324-hydroxylase, Protein KAINESU C, prolactin, a plasma membrane calcium pump, As a main gene which there are an EGF acceptor, the neoplasm necrosis factor alpha, 1alpha,

a 25-hydroxylation vitamin-D3 acceptor, etc., and has received accommodation in control The NADH-DH subunit I, calcitonin, the collagen type I A gamma interferon, a colony SUTIMU rating factor, c-myb, 25-hydroxylation vitamin D 31alpha hydroxylase, fatty-acid joint protein, Interleukin II and III, There are CD-23, a transferrin acceptor, Cytochrome B, ferredoxin, parathyroid hormone (generally abbreviated to PTH), the pre--prow PTH, PTH related protein, protein KAINESU inhibitor, etc. Vitamin D 324-hydroxylase, and male TEOKARU and an osteopontin control region have a vitamin-D3 response array, and although receiving expression control by 1alpha and 25-hydroxylation vitamin D3 is known, it is not shown clearly how [that is what has received the accommodation all the above-mentioned genes minded the vitamin-D3 response array] it is. Although vitamin D 324-hydroxylase was guided when the vitamin D deficiency rat was medicated with 1alpha and 25-hydroxylation vitamin D3, in the kidney and a small intestine, the difference was looked at by the concentration and reaction time, and reactivity was understood that the small intestine is higher. It is vitamin D3 when parathyroid hormone and 1alpha, and 25-hydroxylation vitamin D3 are simultaneously prescribed for the patient. It became clear that induction of 24-hydroxylase is controlled. Moreover, it turned out that this enzyme is discovered to proximal tube with the kidney. It was shown that this enzyme guided by 1alpha and 25-hydroxylation vitamin D3 in a small intestine (1996, endocrinology (Endocrinology), the 137th volume, the 2938th page) has discovered Roy (Roy) etc. to the crypt columnar epithelium and the villus.

[0007] In vitamin D, the reaction (generally referred to as genomicaction of vitamin D) and the other reaction (generally called non-genomic action of vitamin D) by the formation of gene activation are known, and it is suggested that the physiological functions differ. About non-genomic action of vitamin D, the phenomenon of causing the acceleration of absorption and the intracellular increment in intestines calcium in the unit for several minutes is shown. Although the vitamin D metabolite in human plasma changes with Measuring conditions etc., 20 to 70 pg/ml and several half-life hours are known [normal range / in plasma / vitamin D3 / one to 5 ng/ml, and / half-life one day;25-hydroxylation] for vitamin D3 at one to 4 ng/ml, half-life 14 to 21-day;1alpha, and 25-hydroxylation vitamin D3 in ten to 40 ng/ml, and half-life ten to 20-day;24-hydroxylation vitamin D3. It is rare to generate 25-hydroxylation vitamin D3 by liver, and to receive a metabolic regulation, and since it is dependent on the generation in the living body by vitamin D intake or daylight, it becomes the index of vitamin D deficiency nutritionally. On the other hand, 1alpha and 25-hydroxylation vitamin D3 are metabolized by the calcium concentration in blood, and parathyroid hormone concentration with the kidney in response to accommodation, and are maintained at fixed concentration. The low concentration in plasma is seen

with diseases, such as a vitamin-D-dependency II mold, rickets, osteomalacia, chronic renal failure, hypoparathyroidism, hyperthyroidism, and osteoporosis, it is known for diseases, such as secondary hyperparathyroidism and a hypercalcemia, or pregnancy that plasma concentration is rising, and becoming the index of a disease diagnosis is known. Although effectiveness can be nutritionally obtained for lack of vitamin D by taking in the food and pharmaceutical preparation containing vitamin D 2 and vitamin D3, administration of active-vitamin-D pharmaceutical preparation is needed for the rickets whose symptoms are shown with vitamin D resistant rickets, osteomalacia, osteoporosis, a nephrogenic dystrophy, psoriasis, and an antiepiletic agent. Moreover, although active-vitamin-D derivative composition is also performed and many derivatives are compounded, in connection with this, development is accomplished also about research of research of the living thing operation in a cell, and a clinical operation, vitamin D pharmaceutical preparation is used as a remedy of the above-mentioned disease, and many derivatives which are the candidates of a remedy further are also known. Like especially the report (1995, endocrine review (Endocrine Reviews), the 16th volume, the 200th page) of bouillon (Bouillon) etc., research of structure-activity relationship is also often done. Many [the derivative which it progresses also about research of research of the living thing operation in a cell, and a clinical operation, consequently vitamin D pharmaceutical preparation is used as a remedy of the above-mentioned disease, and has become the candidate of a remedy] 24-fluorination vitamin D3 was studied by the detail in it, it becomes clear that a living thing operation of 24 and 24-difluoro-25-hydroxylation vitamin D3 is not a thing with it of 24 and 25-hydroxylation vitamin D3 and a difference, and the function of 1alpha and 25-hydroxylation vitamin D 324-hydroxylase came to be studied more by the detail. Beckmann (Beckman) etc. made it clear [in an in vitro metabolic turnover experiment] (1996, biochemistry (Biochemistry), the 35th volume, the 8465th page) about the many catalyses of this enzyme. 25-hydroxylation-24-oxo-vitamin D3 is metabolized and 25-hydroxylation vitamin D3 is 24-oxo-. - It passes through 23 and 25-hydroxylation vitamin D3, and is 23. - It hydroxylates. - It was shown from the result that it is generated by 24, 25, 26, and 27-tetranol vitamin D3 that this enzyme has not only hydroxylation of the 24th place but many catalyses.

[0008]

[Problem(s) to be Solved by the Invention] A break through of solving the function of a vitamin D 324-hydroxylase gene, the vitamin-D3 metabolic turnover especially in a bone disease, and its operation is an important technical problem on the therapy of bone diseases (for example, a primary and secondary osteoporosis, rickets, osteomalacia, low calcium ****, etc.) and kidney disease (for example, glomerulonephritis, IgA glomerulonephritis,

membranous nephropathy, the glomerulosclerosis, nephrosis, renal failure, etc.). It is an important technical problem on a mechanism break through of the kidney disease and the bone disease which are characterized by furthermore authorizing the improvement effect of kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, or cancer, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, or cancer. Among this inventions, although a transgenic animal is used for the purpose of solving the target gene controlling mechanism of nucleus interoception objects including a break through of the function of vitamin D 324-hydroxylase, vitamin-D3 metabolic turnover research, the examination of the prevention / therapy approach in a bone disease, supply of a gene high manifestation cell, ligand joint research of a vitamin D acceptor, and a vitamin D acceptor etc., the optimal animal is offered. By creation of the transgenic animal of this invention, the superfluous manifestation of vitamin D 324-hydroxylase Inactivation of the gene function to mainly promote the vitamin-D3 metabolic turnover imbalance of the kidney, and to adjust activation mold vitamin D3, Lowering is minded. Kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, A heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, The kidney disease and the bone disease which are characterized by authorizing the improvement effect of Alzheimer's disease or cancer, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, cancer, or the mechanism of those complication was solved. However, now, the foreignness transgenic animal (especially transgenics rat) effective enough as a symptoms model of the bone disease with which such an object can be presented, or kidney disease is not known. Therefore, if it succeeds in production of a transgenic animal which has DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene which serves as a symptoms model, or its mutant alle A break through of a break through of the function of a vitamin D 324-hydroxylase gene, a vitamin-D3 metabolic turnover especially in a bone disease, and its operation is attained. Furthermore, it is thought that a break through of the target gene controlling mechanism of nucleus interoception objects including examination of the prevention / therapy approach in a bone disease, supply of a gene high manifestation cell, ligand joint research of a vitamin D acceptor, and a vitamin

D acceptor etc. is attained.

[0009]

[Means for Solving the Problem] this invention persons succeed in production of a nonhuman mammal which has DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene as a result of inquiring wholeheartedly, in order to solve the above-mentioned technical problem for the first time. The superfluous manifestation of vitamin D 324-hydroxylase mainly promotes the vitamin-D3 metabolic turnover imbalance of the kidney to that this transgenic animal shows the symptoms of kidney disease also unexpectedly, and a pan. Inactivation thru/or lowering of the function of a gene which adjusts activation mold vitamin D3 is minded. Kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, Diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, The kidney disease, the bone disease which are characterized by authorizing the improvement effect of Alzheimer's disease or cancer, Based on a header and these, this invention was completed for showing the symptoms of an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, cancers, or those complication. That is, this invention is a part of nonhuman mammal which has DNA incorporating a (1) foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle, or its living body.;

(2) The animal of the aforementioned (1) publication whose nonhuman mammal is a rabbit, a dog, a cat, a guinea pig, a hamster, a mouse, or a rat, or a part of its living body;

(3) The animal of the aforementioned (1) publication whose nonhuman mammal is a rat, or a part of its living body;

(4) Vector which contains a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle, and may discover this gene in mammalian;

(5) The screening approach of the matter used for prevention and the therapy of the disease resulting from a vitamin-D3 metabolic error which applies an examined substance to a part of nonhuman mammal which has DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle, or its living body, and is characterized by authorizing the improvement effect of the disease resulting from the vitamin-D3 metabolic error of this examined substance;

(6) The screening approach of the aforementioned (5) publication that the disease resulting from a vitamin-D3 metabolic error is kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a

heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, or cancer;
(7) The screening approach of the aforementioned (5) publication that the disease resulting from a vitamin-D3 metabolic error is kidney disease or a bone disease;
(8) Remedy for prevention / therapy of the disease resulting from the vitamin-D3 metabolic error which comes to contain the matter judge that has the improvement effect of the disease which originates in a vitamin-D3 metabolic error by the approach of the aforementioned (5) publication;
(9) Remedy of the aforementioned (8) publication whose disease resulting from a vitamin-D3 metabolic error is kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, or cancer;
(10) Remedy of the aforementioned (8) publication whose disease resulting from a vitamin-D3 metabolic error is kidney disease or a bone disease;
(11) A part of nonhuman mammal which has DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle, or its living body For prevention and the therapy of the disease resulting from a vitamin-D3 metabolic error To the fertilized egg which is made to cross the female rat which prescribed luteinizing hormone 0 [about] thru/or 10IU / individual for the patient with a male rat, and is obtained after prescribing application [for screening the matter used]; and (12) follicle-stimulating hormone 20 [about] thru/or 50IU / individual for the patient DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle is introduced, and the rat of the aforementioned (3) publication characterized by implanting this fertilized egg to a female rat or some the living body's production approaches are offered.
[0010] As opposed to the germinal cell in which the transgenic animal of this invention contains an unfertilized egg, a fertilized egg, a sperm, and its progenitor cell preferably The phase of the embryogenesis in generating of a nonhuman mammal (still more preferably) Are the phase of a single cell or an amphicytula, and, generally it sets before 8 cell terms. a calcium phosphate method, an electric pulse method, the RIPOFE cushion method, a condensation method, a microinjection method, and party Kurgan -- by the transgenics approaches, such as law and the DEAE-dextran method It is created by introducing into the cell aiming at the target foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle. moreover, the gene made into the object by this transgenics approach at a somatic cell, a living body's organ, a tissue cell, etc. -- introducing -- a cell

culture, tissue culture, etc. -- it can also use -- further -- the germinal cell above-mentioned [these cells] and the very thing -- a transgenic animal can also be created by making it unite by the well-known cell fusion method. Moreover, some living bodies of the transgenic animal produced by doing in this way for example, the cell which has DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle -- An organization, an organ, etc. cultivate the cell or organization originating in these. What carried out the passage if needed as "some living bodies of a nonhuman mammal which has DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle" of this invention It can use for the same object as "the nonhuman mammal which has DNA incorporating a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene or its mutant alle" of this invention.

[0011] As target "nonhuman mammal", a cow, Buta, a sheep, a goat, a rabbit, a dog, a cat, a guinea pig, a hamster, a rat, a mouse, etc. are mentioned by this invention. Preferably, it is a rabbit, a dog, a cat, a guinea pig, a hamster, a mouse, or a rat, and Rodentia (Rodentia) are desirable and they are especially rats (Wistar, SD, etc.) and an object animal as an animal used in disease modeling especially with the most desirable rat of a Wistar system. It can use for the object same as a birds animal otherwise as the "nonhuman mammal" which carries out the object of the fowl etc. by this invention. As target "mammalian", Homo sapiens etc. is mentioned other than the above-mentioned "nonhuman mammal" by this invention. As a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene introduced into the target nonhuman mammal, the following etc. are mentioned, for example. The vitamin D 324-hydroxylase of a rat will not be isolated without Daisen (Ohyama) etc. (1989, proceedings OBU National academy OBU Science, the 86th volume, the 8977th page). This enzyme is molecular weight about 55000 protein, and it is P450 of a mitochondrion mold, Daisen (Ohyama) etc. is the assay which used the antibody of vitamin D 324-hydroxylase, and it has succeeded in acquiring the complementary DNA (abbreviated to cDNA) of the magnitude of 3.2kbp(s) eventually. As the structure and the function of this gene, the pro peptide part which consists of amino acid of 35 amino acid ends (generally abbreviated to an amino terminal) after a translation is cut, and it is known that the protein which consists of 479 amino acid and which matured will generate. Moreover, it is also shown clearly that it is equipped with the description of P450 protein since the 462nd cysteine of this protein configurates in the 5th binding site of a hem, and if obtained cDNA is made to discover in a COS cell, since vitamin D 324-hydroxylase activity arises, also in the function, it is checked that it is cDNA of vitamin D 324-hydroxylase. Besides the vitamin D 324-hydroxylase cDNA of the above-mentioned rat Human vitamin D 324-hydroxylase cDNA (a chain (Chen) etc.) 1993

Proceedings OBU National Academy OBU Science, The 90th volume, the 4543rd page, vitamin D 324-hydroxylase cDNA of a mouse (Ito (Itoh) etc.) 1995, biotechnology KEMIKA E Biotechnology FIJIKA AKUTA (Biochemica et Biophysica Acta), The 1264th volume, the 26th page, vitamin D 324-hydroxylase cDNA of a guinea pig (Daisen (Ohyama) etc.) DNA arrays, such as 1996, the Japanese Society for Bone and Mineral Research journal, the 14th volume, and the 112nd page, are well-known, and you may use as a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene which introduces the vitamin D 324-hydroxylase cDNA of which animal species into the target nonhuman mammal. Specifically as a mutant alle of the foreignness 25-hydroxylation vitamin D 324-hydroxylase gene of this invention, what variation (for example, mutation etc.) produced, and the gene which addition of a base, the deficit, the permutation to other bases, etc. produced are mentioned to the DNA array of the original foreignness 25-hydroxylation vitamin D 324-hydroxylase gene. As a result of addition of this base, a deficit, and the permutation to other bases, it may be desirable to make it vary so that a permutation, addition, or a deficit may arise in the amino acid sequence which constitutes 25-hydroxylation vitamin D 324-hydroxylase to 1 thru/or five amino acid (preferably 1 or two pieces), and as long as it is the variation which does not lose the function of the original 25-hydroxylation vitamin D 324-hydroxylase, more specifically, you may be which variation.

[0012] The foreignness 25-hydroxylation vitamin D 324-hydroxylase gene in this invention or its mutant alle may be the thing of the nonhuman mammal made into the object of installation or a manifestation, congener, or which mammalian origin of a different kind. If it hits making this gene introduce into an object animal, generally it is advantageous to use as gene constructs (an example, vector, etc.) connected with the lower stream of a river of the promotor who is made discovered in the cell of the target animal and deals in the gene concerned. When making a human 25-hydroxylation vitamin D 324-hydroxylase gene specifically introduce, the various mammalians (a rabbit --) which have a Homo sapiens 25-hydroxylation vitamin D 324-hydroxylase gene and a 25-hydroxylation vitamin D 324-hydroxylase gene with high homology It originates in a dog, a cat, a guinea pig, a hamster, a rat, a mouse, etc. (preferably rat etc.). On the lower stream of a river of the various promotors who are made discovered and deal in a human 25-hydroxylation vitamin D 324-hydroxylase gene The transgenics nonhuman mammal which carries out the high manifestation of the Homo sapiens 25-hydroxylation vitamin D 324-hydroxylase gene made into the object can be created by carrying out the microinjection of the vector which connected this gene to the fertilized egg (for example, rat fertilized egg) of the target nonhuman mammal. As a 25-hydroxylation vitamin D 324-hydroxylase gene expression vector, animal viruses, such as retroviruses, such as bacteriophages, such as

a plasmid of the Escherichia coli origin, a plasmid of the Bacillus-subtilis origin, a plasmid of the yeast origin, and lambda phage, and a Moloney leukemia virus, a vaccinia virus, or a baculovirus, etc. are used. Especially, the plasmid of the Escherichia coli origin, the plasmid of the Bacillus-subtilis origin, or the plasmid of the yeast origin is used preferably, and especially the plasmid of the Escherichia coli origin is desirable.

[0013] As a promotor who performs gene expression accommodation of a 25-hydroxylation vitamin D 324-hydroxylase gene for example, a virus (a cytomegalovirus and a MORONI leukemia virus --) The promotor of the gene originating in JC virus, a milk oncogenic virus, etc., various mammalians (Homo sapiens, a rabbit, a dog, a cat, a guinea pig, and a hamster --) The gene originating in birds (fowl etc.), such as a rat and a mouse for example, albumin, endothelin, osteocalcin, and muscle creatine kinase -- A collagen I-beam and II mold, a cyclic AMP dependence protein kinase betaI subunit, An atrium natriuresis sex factor, a dopamine-beta-hydroxylase, a neurofilament light chain, Metallothioneins I and IIA, METARO proteinase 1 organization inhibitor, Although promotors, such as smooth muscle alpha actin and polypeptide chain elongation factor 1alpha (EF-1alpha), beta actin, alpha and beta myosin heavy chain, myosin light chains 1 and 2, myelin basic protein, a blood serum amyloid P component, and renin, etc. are mentioned The MORONI leukemia virus promotor who can carry out a high manifestation in the whole body preferably, Homo sapiens, a fowl beta actin promotor, etc. can be used. In order to make a bone unique target discovered with Homo sapiens, a rat, a mouse, etc., the promotor of an osteocalcin gene and an S TEROJIEN acceptor gene by whom it is known that it will be discovered to a bone is effective.

[0014] as for the above-mentioned vector, it is desirable to have the array (poly A -- generally called TAMINETA) which ends the imprint of messenger RNA made into the object in transgenics mammalian, for example, gene expression can be operated using the array of each gene of the virus origin, various mammalians, and the birds origin. Preferably, SV40 TAMINETA of a simian virus etc. is used. In addition, it is the object which carries out the high manifestation of the target gene further, and is 3'lower stream of a river of between 5'upstream, the promoterregions, and the translation fields of promoterregion, or a translation field in a part of intron of the splicing signal of each gene, an enhancer field, and an eukaryon gene. Connecting is also possible by the object.

[0015] The translation field of vitamin D 324-hydroxylase is acquirable from RNA originating in liver, the kidney, and fibroblast, using the complementary DNA prepared by the well-known approach as a raw material, using all or a part of genomic DNA originating in DNA originating in the liver of various mammalians (a rabbit, a dog, a cat, a guinea pig, a hamster, a rat, mouse, etc.),

the kidney, fibroblast, etc., and various commercial genomic DNA libraries as a raw material. Moreover, the complementary DNA prepared by the well-known approach from RNA originating in a patient's fibroblast can also be used for a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene as a raw material. Moreover, the translation field which varied by the point mutation inducing method etc. is also producible using the translation field of the vitamin D 324-hydroxylase obtained from an above-mentioned cell or an above-mentioned organization. Each of these is ingredients available to a transgenic animal. The above translation field can produce DNA incorporating a 25-hydroxylation vitamin D 324-hydroxylase gene by the usual gene engineering-technique made to connect with the aforementioned promotor's lower stream of a river (preferably upstream like the termination section of an imprint) as gene constructs (an example, vector, etc.) which may be discovered in an introductory animal.

[0016] Installation of the 25-hydroxylation vitamin D 324-hydroxylase gene in an amphicytula phase is secured so that it may exist in all the germinal cells of object mammalian, and somatic cells. In the germinal cell of the creation animal after transgenics, as for a 25-hydroxylation vitamin D 324-hydroxylase gene existing, all the animals of the progeny of a creation animal mean that a 25-hydroxylation vitamin D 324-hydroxylase gene exists in all the germinal cell and somatic cells. The descendant of this kind that inherited the gene of animal has a 25-hydroxylation vitamin D 324-hydroxylase gene in all that germinal cell and somatic cells.

[0017] By acquiring the homozygote animal which has an introductory gene in both homologues, and crossing the animal of this sex, it can check all descendants holding this gene to stability, and having this gene superfluously, and a propagation passage can be carried out in the usual breeding environment. A different gene from the gene of the internality which the animal for transgenics has (preferably) The 25-hydroxylation vitamin D 324-hydroxylase gene of the foreignness which is the gene which does not hold the intron The fertilized egg used in case it introduces into the fertilized egg of an object nonhuman mammal (it is Wistar especially preferably preferably, such as a rat, rat of a system etc.) or its ancestor It is obtained by making a male nonhuman mammal (it being Wistar especially preferably preferably, such as a male rat, male rat of a system etc.) and a female nonhuman mammal (it being Wistar especially preferably preferably, such as a female rat, female rat of a system etc.) of the same kind cross. Although a fertilized egg is obtained also by natural mating, after adjusting artificially the sexual cycle of a female nonhuman mammal (it is Wistar especially preferably preferably, such as a female rat, female rat of a system etc.), a male nonhuman mammal (it is Wistar especially preferably preferably, such as a female rat, female rat of a system etc.) and the approach of making it cross

are desirable. as an approach of adjusting the sexual cycle of a female nonhuman mammal artificially, although introduction follicle-stimulating hormone (pregnant mare serum gonadotropin -- it generally abbreviates to PMSG) and the method of prescribing luteinizing hormone (Homo sapiens chorionic gonadotropin -- it abbreviating to hCG generally) for the patient for example, by abdominal cavity injection etc. are subsequently desirable, the dose of desirable hormone and administration spacing change with classes of nonhuman mammal, respectively, for example. Moreover, Wister When using the rat of a system, the thing 8 weeks old or more bred for about one week on about 12-hour ** term conditions (for example, 7:00 - 19:00) is desirable. the approach of obtaining a fertilized egg when a nonhuman mammal prescribes luteinizing hormone for the patient follicle-stimulating hormone administration and about 48 hours after and makes it usually crossing with a male rat in the case of a female rat (preferably female rat of Wistar system) -- desirable -- the dose of follicle-stimulating hormone -- about 20 - about 50 IU / individual -- desirable -- the dose of about 30 IU / individual, and luteinizing hormone -- about 0 - about 10 IU / individual -- they are about 5 IU / individual preferably. After a foreignness 25-hydroxylation vitamin D 324-hydroxylase gene is introduced into the obtained fertilized egg by the above-mentioned approach, it is artificially transplanted and implanted to a female nonhuman mammal, and the nonhuman mammal which has DNA incorporating a foreignness gene is obtained. Moreover, the method of transplanting and implanting artificially the fertilized egg obtained in the fertility by the pseudopregnancy female nonhuman mammal by which induction was carried out is [after prescribing luteinizing hormone releasing hormone (it generally abbreviates to LHRH), or its analog for the patient] also desirable by making it cross with male Homo sapiens mammalian. A male nonhuman mammal and the stage made to cross change with classes of nonhuman mammal after LHRH or the dose of the analog, and its administration, respectively. A nonhuman mammal in the case of a female rat (it is Wistar preferably female rat of a system) Usual, LHRH or its analog (for example, [3 and 5-Dil-Tyr5]-LH-RH, [Gln8]-LH-RH, [D-Ala6]-LH-RH, [des-Gly10]-LH-RH, [D-His(Bzl)6]-LH-RH, and they) [ ] making it cross with a male rat on about the 4th, after prescribing Ethylamide etc. for the patient -- desirable -- the dose of LHRH or its analog -- usually -- about 10-60microg / individual -- they are about 40microg / individual preferably. Moreover, it is desirable to use the obtained fertilized egg for the pseudopregnancy female nonhuman mammal by which induction was carried out to the approach of adjusting artificially the sexual cycle of the above-mentioned female nonhuman mammal, and acquiring a fertilized egg in the fertility combining the approach of transplanting and implanting artificially.

[0018] The nonhuman mammal into which the 25-hydroxylation vitamin D

324-hydroxylase gene of this invention was introduced When the 25-hydroxylation vitamin D 324-hydroxylase gene is made to carry out a high manifestation and promotes the function of the 25-hydroxylation vitamin D 324-hydroxylase gene of internality Eventually A hypercalcemia, hyperparathyroidism, rickets, osteomalacia, Kidney diseases, such as bone diseases, such as osteoporosis and osteopenia, and glomerulonephritis, chronic nephritis, and renal failure, Furthermore it may become an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, or cancer, and can use as those symptoms model animals. A mouse, a rat, etc. of this invention are used. For example, a hypercalcemia, A bone disease and glomerulonephritis, such as hyperparathyroidism, rickets, osteomalacia, osteoporosis, and osteopenia, To kidney diseases, such as chronic nephritis and renal failure, and a pan, an articular disease, a lung disease, hyperlipidemia, Arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, It is possible to screen the candidate compound aiming at a break through of symptoms mechanisms, such as an endocrinologic disease, Alzheimer's disease, or cancer, examination of the prevention / therapy approach of these diseases, and prevention and remedy researches and developments of these diseases. Moreover, as availability of the nonhuman mammal into which the 25-hydroxylation vitamin D 324-hydroxylase gene was introduced of this invention A 25-hydroxylation vitamin D 324-hydroxylase gene high manifestation mouse A hypercalcemia, hyperparathyroidism, rickets, osteomalacia, osteoporosis, Kidney diseases, such as bone diseases, such as osteopenia, and glomerulonephritis, chronic nephritis, and renal failure, Becoming the experimental model which furthermore solves the symptoms mechanism in kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, or cancer is also mentioned. In that case, the utilization as an experimental model which the function of 25-hydroxylation vitamin D 324-hydroxylase in the living body will be solved, and solves the gene regulatory mechanism of the vitamin D receptor which is a receptor in a nucleus further is also expected.

[0019] It is also possible to use the transgenics mammalian of the above this invention as a source of a cell for tissue culture. Moreover, it is also analyzable about relevance with the transcription factor of a complicated operation of the receptor in a nucleus by analyzing the protein organization which did the direct method of analysis of DNA or RNA under organization of the trans-genic mice of this invention, or was discovered by the gene, for

example. Or the function of a cell in which cultivate the cell of the organization which has a gene with a normal structure culture technique, and use these, for example, culture originates in a difficult organization generally [osteoblast, an osseous tissue origin cell like an osteoclast, etc.] can also be studied. Furthermore, selection of drugs which raise the function of a cell is also possible by using the cell. Moreover, if there is a high manifestation cell strain, it is that it is also possible to carry out isolation purification of the vitamin D 324-hydroxylase at a large quantity and to produce the antibody from there.

[0020] The transgenics nonhuman mammal of this invention is a model available to the screening trial of prevention and remedies, such as kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, allergosis, an endocrinologic disease, Alzheimer's disease, or cancer. Especially, the transgenics nonhuman mammal of this invention is a model more nearly available than having renal dysfunction to the screening trial of the prevention and the remedy of kidney disease. Moreover, since this transgenics nonhuman mammal has the symptom of bone quantity reduction, it is a model available to the screening trial of osteoporosis prevention and remedies, such as vitamin-D3 pharmaceutical preparation, for example. Although the trouble relevant to the following specific difference exists in the bone disease models (a rat, mouse, etc.) used conventionally, the trouble relevant to such the specific difference is also conquered, and the transgenics nonhuman mammal of this invention is considered to be an effective animal also from such a viewpoint.

(1) In a rat, a peak is seen like Homo sapiens by the bone quantity change by aging.

(2) In the rat, change by aging of the amount of bone components is often investigated.

(3) Compared with a mouse, as for alkaline phosphatase and tartaric-acid resistance acid phosphatase (generally abbreviated to TRAP) which are the biochemical marker of osteogenesis and osteoclasis, a significant difference is easy to be detected by the rat. Compared with a mouse, a rat tends [furthermore] to study bone gestalten, such as bone morphometry.

(4) In the fundamental research of the bone disease to current, and screening of remedy development, the example which is using the rat rather than the mouse is a generality.

[0021] As a kidney disease model, the following viewpoints to effectiveness is still higher.

(1) The nephrectomy mouse and the rat are used well, and although it is the model which causes lowering of a kidney function, since it is hard to say that the symptoms of kidney disease are reflected, the transgenics rat of this

invention shows the same progress as human kidney disease to the ability to use only for screening and assessment of remedy development of a part.
(2) As for the transgenics rat of this invention, high proteinuria is detected at a juvenile term, and the amount is in the inclination of lifting with ****. The symptoms of kidney disease and relation with a biochemical marker are also reflected.
(3) A normal rat and a difference are not seen but it is easy to use the weight increase and fertility of a transgenics rat of this invention.
(4) Although the model of glomerulonephritis and IgA glomerulonephritis does not exist, approximate the pathology image of the transgenic animal of this invention to it.
(5) The increment in proteinuria is seen also in a hyperlipidemia model rat SHC system, an obesity model rat Wistar Fatty system, etc., and these existing models die of a 25-30-week ** grade. However, the transgenics rat of this invention can be used also as a model which kidney disease made critical, and is viable to old age.
(6) Since the transgenics rat of this invention shows the symptoms of kidney disease at a juvenile term, it goes on with **** and the osteoporosis's bone disease is shown, the effectiveness as a model of the complication patient of kidney disease and a bone disease is high.
[0022] A part of nonhuman mammal which has DNA incorporating the foreignness 25-hydroxylation vitamin D 324-hydroxylase gene of this invention or its mutant alle, or its living body is useful by applying to an examined substance also as an experimental model for screening the matter (drug) used for prevention and the therapy of the disease resulting from a vitamin-D3 metabolic error by authorizing an improvement of the disease resulting from the vitamin-D3 metabolic error of this examined substance. As ** "the disease resulting from a vitamin-D3 metabolic error", kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, or cancer is raised, for example (preferably kidney disease or a bone disease etc.). For example, as a test compound, the organization (for example, mouse, rat, Buta, cow, sheep, ape, Homo sapiens, etc.) extract of a homeothermic mamal, cell culture supernatant liquid, etc. are used else [, such as a well-known synthetic compound, a peptide, and protein]. For example, the transgenics nonhuman mammal of this invention is medicated with this organization extract, cell culture supernatant liquid, or its purification object. Or a part of the living body (an example, a cell, an organization, organ, etc.) is added thru/or contacted. By authorizing an improvement operation thru/or effectiveness, such as kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a

heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, allergosis, an endocrinologic disease, Alzheimer's disease, or cancer The drugs for prevention / therapy of this disease (for example, a peptide, protein, a nonpeptidic compound, a synthetic compound, a fermentation product, etc.) can be screened. By the screening approach of this invention, moreover, kidney disease, a bone disease, an articular disease, A lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, The matter (25-hydroxylation vitamin D 324-hydroxylase inhibitor etc.) judge that has improvement effects, such as an infectious disease, allergosis, an endocrinologic disease, Alzheimer's disease, or cancer Kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, Diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, It is useful because of prevention and therapies, such as Alzheimer's disease or cancer, and this is used. It is possible to prepare the remedy for prevention / therapy of diseases, such as kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, allergosis, an endocrinologic disease, Alzheimer's disease, or cancer, according to a well-known pharmaceutical preparation means. The array number of the array table of this invention shows the following arrays.

[-- array number: -- 1] -- PCR (polymerase chain reaction) performed in the below-mentioned example 2 -- the base sequence of the primer used for law is shown.

[-- array number: -- 2] -- PCR (polymerase chain reaction) performed in the below-mentioned example 2 -- the base sequence of the primer used for law is shown.

[-- array number: -- 3] -- PCR (polymerase chain reaction) performed in the below-mentioned example 3 -- the base sequence of the primer used for law is shown.

[-- array number: -- 4] -- PCR (polymerase chain reaction) performed in the below-mentioned example 3 -- the base sequence of the primer used for law is shown.

[-- array number: -- 5] -- PCR (polymerase chain reaction) performed in the below-mentioned example 4 -- the base sequence of the primer used for law is shown.

[-- array number: -- 6] -- PCR (polymerase chain reaction) performed in the below-mentioned example 4 -- the base sequence of the primer used for law is shown.

[0023]

[Embodiment of the Invention] When displaying a base, amino acid, etc. by the

code in this application description, it is IUPAC-IUBCommision on Biochemical Nomenclature. Based on the code to depend or the common use code in the field concerned, the example is given to a degree.
DNA : deoxyribonucleic-acid RNA : Ribonucleic-acid A : Adenine T : Thymine G : Guanine C : Cytosine [0024]
[Example] Although an example is given to below and this invention is more concretely explained to it, it cannot be overemphasized that this invention is not limited to these.
[0025] The construction MORONI leukemia virus promotor (MLV-LTR) of plasmid pMLV-LTR-VitaminD324-Hydroxylase who has a 25-hydroxylation vitamin D 324-hydroxylase gene on the lower stream of a river of the gene regulatory region of example 11 Moloney leukemia virus used the plasmid pIRAO1 of the plasmid pYJ1 origin indicated by GOFU (Goff) etc. (1980, a cel (Cell), the 22nd volume, the 777th page). TAMINETA (SV40) used the terminator contained in the plasmid pIRA01 of the plasmid pcVD1 origin indicated in; Okayama (1983, molecular , and cellular biology (Mol.Cell.Biol.), the 3rd volume, the 280th page) (Okayama), such as Okayama (Okayama), etc. (1982, molecular , and cellular biology (Mol.Cell.Biol.), the 2nd volume, the 161st page). The vector for a rat 25-hydroxylation vitamin D 324-hydroxylase cDNA manifestation was built as follows. Restriction enzymes EcoRI and ScaI cut pUC19-24-hydroxylase (it sells in lots from Professor Showa University department-of-dentistry Tatsuo Suda and Assistant professor Hiroshima University science department Yoshihiko Oyama) of a publication (1991, FEBUSU Letters (FEBS Lett.), the 278th volume, the 195th page) to Daisen and others (Ohyama) etc., and the fragment of 3.2kbp(s) containing rat 25-hydroxylation vitamin D 324-hydroxylase cDNA was obtained. EcoRI-PmacI fragment (2.1kbp) 100ng of the pUC19-24-hydroxylase origin which cuts this fragment by PmacI and is obtained was processed by calf small intestine alkaline phosphatase (TAKARA SHUZO CO., LTD. make), and dephosphorization of 5' edge was performed. Next, restriction enzymes SmaI and EcoRI cut the multi-cloning site inserted in LacZ of commercial pBluescript II KS (+), and the SmaI-EcoRI fragment (2.9kbp) of the pBluescript II KS (+) origin was obtained. Using the TAKARA ligation kit (TAKARA SHUZO CO., LTD. make), the EcoRI-PmacI fragment (2.1kbp) of the pUC19-24-hydroxylase origin and 16 degrees C (2.9kbp) of SmaI-EcoRI fragments of the pBluescript II KS (+) origin are processed for 60 minutes, they were combined, the transformation of Escherichia coli JM 109 (NIPPON GENE, Inc. make) was carried out using this reaction mixture, and the ampicillin resistant strain was obtained. Plasmid DNA was collected from this transformant (Escherichia coli JM109/pBluescript II KS(+)-24-hydroxylase), restriction enzyme cutting was performed, it checked that the EcoRI-PmacI fragment of the pUC19-24-hydroxylase origin was combined in the forward

direction in the SmaI-EcoRI fragment of the pBluescript II KS (+) origin, and plasmid pBluescript II KS(+)-24-hydroxylase (5.0kbp) was obtained. The rearrangement which can be detected was not included when this gene construct was inspected by multiplex restriction enzyme cutting. Next, the EcoRI-BamHI fragment which cuts pBluescript II KS(+)-24-hydroxylase with restriction enzymes EcoRI and BamHI, and contains rat 25-hydroxylation vitamin D 324-hydroxylase cDNA was acquired. The EcoRI-BglII fragment which, on the other hand, cuts pIRA01 which is a MLV-LTR content vector by EcoRI and BglII, and contains MLV-LTR was acquired. Using the TAKARA ligation kit (TAKARA SHUZO CO., LTD. make), the EcoRI-BamHI fragment containing the rat 25-hydroxylation vitamin D 324-hydroxylase cDNA of that account of Gokami (pUC19-24-hydroxylase origin) and 16 degrees C of EcoRI-BglII fragments containing MLV-LTR are processed for 60 minutes, they were combined, the transformation of Escherichia coli JM 109 (NIPPON GENE make) was carried out using this reaction mixture, and the ampicillin resistant strain was obtained. Plasmid DNA was collected from this transformant (Escherichia coli JM109/pMLV-LTR-VitaminD324-hydroxylase), restriction enzyme cutting was performed, it checked that the EcoRI-BamHI fragment of the pBluescript IIKS(+)-24-hydroxylase origin was combined in the forward direction in the EcoRI-BglII fragment of the pIRA01 origin, and plasmid pMLV-LTR-VitaminD324-hydroxylase (6.2kbp) was obtained. The rearrangement which can be detected was not included when this gene construct was inspected by multiplex restriction enzyme cutting. This plasmid construction drawing is shown in drawing 1 .

[0026] 2) It is the female rat Wistar as an object for creation egg gathering of the transgenics rat containing the gene fusion object which the 25-hydroxylation vitamin D 324-hydroxylase gene combined with the lower stream of a river of the gene regulatory region of a Moloney leukemia virus. A system is purchased by 8 weeks old. The 9-weeks old animal bred for one week on the 12-hour ** term conditions of 7:00-19:00 is used. Will inject 11:00 with follicle-stimulating hormone (pregnant mare serum gonadotropin) (30IU / individual) first for the 1st day intraperitoneal, and it will breed on the same conditions. 3 Eye 11:00 a day is injected with luteinizing hormone (Homo sapiens chorionic gonadotropin) (5 IU / individual) intraperitoneal, and it is the male rat Wistar. The individual after 10 weeks old of systems, and 17:00 It was made to live together and cross by 1:1. The vaginal plug check of the female rat which 9:00 was made to cross for the 4th day was performed, and egg gathering was started after slaughtering the individual which carried out the vaginal plug check from 13:30. The pronucleus formation egg was chosen by the fertilized egg. ClaI cut the above-mentioned plasmid pMLV-LTR-VitaminD324-Hydroxylase from 14:30, and it prepared to 10micro g-100microg [/ml] concentration, and poured in to the male pronucleus of

the Wistar system rat egg of the unicellular term fertilized while observing the 1-2microl under the microscope. It cultivates by the HER culture medium, and after checking 2 cell germ to 13:30 for the 5th day, you made it transplanted and implanted on the oviduct of the female Wistar system rat of pseudopregnancy according to the approach indicated by Wagner and others (Wagner) (I98I., proceedings OBU National academy OBU Science, the 78th volume, the 5016th page). The female Wistar system rat (11 weeks old or subsequent ones) of pseudopregnancy has lived together, and was made to cross by the individual after the male Zucker lean system which carried out subcutaneous injection (40microg / individual) of 13:00 gonadotropic hormone releasing hormone (Luteinizing Hormone-Releasing Hormone) or the analog of those on the 0th, and carried out vasoligature to 17:00 on the 4th, or 12 weeks of Wistar system rats, and 1:1. The vaginal plug check of the female rat made to cross was performed to 9:00, and it was used for it for the above-mentioned object on the 5th.

[0027] It examined by the polymerase chain reaction method using DNA which reached 3 weeks old of gene analyses of an example 225-hydroxylation vitamin D 324-hydroxylase transgenics rat, out of which it came and which was extracted from the tail of offspring. That is, polymerase chain reaction (PCR) was performed using the primer 1 (5'-AGGCTGTGCTGCTAATGTCAA-3': array number 1) of 21 MA in rat vitamin D 324-hydroxylase cDNA, and the primer 2 (5'-AAGAGTGGGGGTCAGAGTTCG-3': array number 2) of 21 MA in rat vitamin D 324-hydroxylase cDNA. What diluted 1micro of tail DNA preparation objects I with sterilized water 50 times was used for the substrate, first, for 3 minutes, -> with a minute [1 minute] of -> with a seconds [30 seconds] of 94 degrees C 65 degrees C 72 degree-C cycle of 1 minute was repeated 25 times continuously, electrophoresis of the reactant was carried out through agarose GTG (FMC biotechnology product company make) gel 1.0%, and 94 degrees C of rats as which the DNA band of the magnitude of 783bp is regarded were sorted out once. As a result of analyzing the offspring rat of a total of 141 individuals, PCR positivity individuals were six individuals. One individual was a death individual among those. About these PCR positivity 5 individual, the 940bp fragment which cut the JIKOKISHI genin-DNA probe (it is the indicator by the RIBOPU lobe method) of 600bp(s) or the (2) rat vitamin D 324-hydroxylase cDNA which includes a (1) rat vitamin D 324-hydroxylase gene sequence using a tail DNA preparation object with restriction enzymes XbaI and KpnI was further analyzed by the Southern hybridization method using the probe which carried out the indicator by the random prime method by 32 P-dCTP. In each case, PstI/BamHI cut DNA from a tail and the fragment by which 32P indicator was carried out was examined as a probe. DNA for analysis extracted DNA from about 1cm

fragment of a tail by the approach indicated by Hogan and others (1986, MANIPIYU rating THE mouse embryo (cold spring harbor laboratory)). It washed once in 70% ethanol, and the obtained nucleic-acid pellet was dried, and 10mM tris (pH 8.0) of 200microl and 1mM EDTA were made to re-suspend. Furthermore, it moved to the nylon filter by the approach of the PCR positivity of these five animals, and the pseudopositive individual DNA which cut 10microg thoroughly with restriction enzymes XhoI and ClaI, respectively, and performed electrophoresis through agarose GTG gel 1.0%, and was indicated by the Southern (Southern) (1975, journal molecular biology, the 98th volume, the 503rd page). When a RIBOPU lobe was used, hybridization was performed by having used this filter as the probe overnight, 2xSSC and 0.1%SDS washed twice at the room temperature after that, and then 0.1xSSC(s) and 0.1%SDS washed twice at 68 degrees C. Since the signal was seen in four animals in the location of 1.8kbp(s) among five authorized animals as a result of this Southern hybridization method, having held the vitamin D 324-hydroxylase gene into which four creation rats were injected was shown. On the other hand, when the probe which carried out the indicator by the random prime method was used, using the tail DNA preparation object, restriction enzymes NsiI and Cla I cut 10micro [of each DNA] g thoroughly, electrophoresis was performed through agarose GTG gel 1.0%, and it moved to the nylon filter like the above. 4xSSC(s) and 50% formamide and 5xDenhart liquid and 50microg [/ml] salmon sperm DNA and 0.2%SDS pre hybridization liquid performed hybridization for this filter at 65 degrees C after 2-hour processing, a probe, and overnight, 1xSSC and 0.1%SDS solution washed 4 times for 15 minutes at the room temperature after that, and then 0.1xSSC(s) and 0.1%SDS washed 4 times for 15 minutes at 68 degrees C. Since the signal was seen in one animal in the location of 3.0kbp(s) among five authorized animals as a result of this Southern hybridization method, having held the vitamin D 324-hydroxylase gene into which one creation rat was injected was shown. From the result of the above Southern analysis, 25-hydroxylation vitamin D 324-hydroxylase transgenics was checked by a total of five individuals. It was checked from the above result that transgenics rats are individual number R9121-7 (male), R12123-6 (female), R12121-7 (female), R11293-5 (female), and 5 of R11293-9 (female) individuals. Moreover, as for R10175-9 (male), installation of a gene was checked by only PCR. (A table 1 and drawing 2 - 3 reference)

[A table 1]

ラットpMLV-LTR-VitaminD3-24Hydroxylaseの注入による遺伝子転移ラットの作[

遺伝子	実験番号	卵数		ラット数		
		注入卵数	移植胚数	産仔数	PCR検定個体数	
pMLV-LTR-VitaminD3-24Hydroxylase	37	110	63	18	18	
	38	265	60	17	30	
	39	245	97	21	21	
	40	220	98	36	36	
	41	274	61	24	24	
	42	126	57	18	18	
	計		436	142	141	

[0028] As regulatory region of the Cronin glut chromosomal gene of the promotor of an example 31 rat osteocalcin gene, the promotor of a rat osteocalcin gene LeAnn (Lian) (1989 and proceedings OBU National academy OBU Science --) etc. The 86th volume and based on the data of a page [1143rd] base sequence, the primer 3 (5'-TGAGGACATTACTGACCGCTCCTT-3': array number 3) of 24 MA in rat osteocalcin gene genomic DNA, And it obtained by the PCR method using the primer 4 (5'-AGTTGCTGTGTGGGACTTGTCTGT-3': array number 4) of 24 MA in rat osteocalcin gene genomic DNA. It is this obtained fragment TA-CloningKit Cloning was used and carried out. The base sequence was checked with the conventional method by the DNA sequencer by the ABI company.

[0029] 2) Construction TAMINETA (SV40) of plasmid pOsteocalcin-VitaminD3-24hydroxylase which has a rat 25-hydroxylation vitamin D 324-hydroxylase gene on the lower stream of a river of the regulatory region of a rat chromosomal gene Okayama (Okayama) (1983, molecular , and cellular biology (Mol.Cell.Biol.) --) etc. the 3rd volume and the 280th -- page; Okayama (Okayama) (1982, molecular , and cellular biology (Mol.Cell.Biol.) --) etc. The terminator contained in the plasmid pIRA01 of the 2nd volume and the plasmid pcVD1 origin indicated by the 161st page was used. Plasmid pPCRII-Osteocalcin promoter in which the rat Osteocalcin gene expression control region was inserted was cut by HindIII and XbaI, and the fragment of 600bp was obtained. On the other hand, pUC118 was cut by HindIII and XbaI, using the TAKARA ligation kit (TAKARA SHUZO CO., LTD. make), the 16 degrees C of the above-mentioned fragments are processed for 60 minutes, they were combined, the transformation of Escherichia coli JM 109 (NIPPON GENE make) was carried out using this reaction mixture, and the ampicillin resistant strain was obtained. Plasmid DNA is collected from this transformant (Escherichia coli JM109/pUC118-Osteocalcin promoter), restriction enzyme cutting is performed, and it checks that the fragment of 600bp is combined with pUC118, and is plasmid

pUC118-Osteocalcin. promoter (3.8kbp) was obtained. Next, restriction enzymes ScaI and XhoI cut it and the ScaI-XhoI fragments of 2.0kbp were collected. Plasmid pMLV-LTR-VitaminD324-hydroxylase (6.2kbp) obtained in the example 1 was cut by ClaI, and the fragments containing VitaminD324-hydroxylase cDNA of 3.2kbp were collected. pUC119 was cut by AccI, using the fragment and the TAKARA ligation kit (TAKARA SHUZO CO., LTD. make) which contain VitaminD324-hydroxylase cDNA of 3.2kbp(s) for this fragment, process 16 degrees C for 60 minutes, it was made to join together, the transformation of Escherichia coli JM 109 (NIPPON GENE, Inc. make) was carried out using this reaction mixture, and the ampicillin resistant strain was obtained. Plasmid DNA was collected from this transformant (Escherichiacoli JM109/pUC119-MLV-24-hydroxylase), restriction enzyme cutting was performed, it checked that the fragment of 1500bp was combined with pUC119, and plasmid pUC119-MLV-24-hydroxylase (6.2kbp) was obtained. Then, it cut by ScaI and XhoI and the ScaI-XhoI fragment of 3.8kbp was acquired, using the TAKARA ligation kit (TAKARA SHUZO CO., LTD. make), this fragment and the 16 degrees C of the above-mentioned ScaI-XhoI fragments of 2.0kbp(s) are processed for 60 minutes, they were combined, the transformation of Escherichia coli JM 109 (NIPPON GENE, Inc. make) was carried out using this reaction mixture, and the ampicillin resistant strain was obtained. Plasmid DNA was collected from this transformant (Escherichia coli JM109/pUC-Osteocalcin-VitaminD324-hydroxylase), restriction enzyme cutting was performed, it checked that the fragment of 1500bp was combined in the forward direction by pUC119, and plasmid pUC-Osteocalcin-VitaminD324-hydroxylase (5.8kbp) was obtained (drawing 4).

[0030] This gene expression vector that introduced the 25-hydroxylation vitamin D 324-hydroxylase gene into the lower stream of a river of the creation Osteocalcin promotor of the transgenics rat containing the gene fusion object which the 25-hydroxylation vitamin D 324-hydroxylase gene combined with the lower stream of a river of the regulatory region of an example 41 rat chromosomal gene was poured into 78 Wistar/crj rat fertilized eggs, and was transplanted to five pseudopregnancy rats. Consequently, transgenics was not checked although the offspring of 17 animals was obtained. HindIII and ScaI cut the above-mentioned plasmid pUC-Osteocalcin-VitaminD324-hydroxylase, and it prepared to 10micro g-100microg [/ml] concentration, and poured in to the male pronucleus of the Wistar system rat egg of the unicellular term fertilized while observing the 1-2microl under the microscope. Subsequently, the poured-in egg was transplanted and implanted to the oviduct of the female Wistar system rat of pseudopregnancy according to the approach indicated by Wagner and others (Wagner) (1981., proceedings OBUNASHONARU academy OBU Science, the

78th volume, the 5016th page). These eggs are obtained by mating of a Wistar system rat. A Wistar system rat was made to grow using a commercial item (Japanese Clare, Inc.) until it hatched an egg in a breeding female rat. the female Wistar system rat (11 weeks old or subsequent ones) of pseudopregnancy has lived together, and was made to cross by the individual after the male Zucker lean system which carried out subcutaneous injection (40microg / individual) of 13:00 gonadotropic hormone releasing hormone (Luteinizing Hormone-Releasing Hormone -- generally abbreviated to LHRH), or the analog of those on the 0th, and carried out vasoligature to 17:00 on the 4th, or 12 weeks of Wistar system rats, and 1:1 The vaginal plug check of the female rat made to cross was performed to 9:00, and it was used for it for the above-mentioned object on the 5th.

[0031] 2) It examined by the polymerase chain reaction method using DNA which reached 3 weeks old of analysis of the rat which introduced the rat gene, out of which it came and which was extracted from the tail of offspring. That is, the polymerase chain reaction method was performed using the primer 5 (5'-CTGTCTTCTTTCAACCTGGAT-3': array number 5) of 21 MA in rat 25-hydroxylation vitamin D 324-hydroxylase cDNA, and the primer 6 (5'-TTAGAGTTCTGTGGGGCATTC-3': array number 6) of 21 MA in rat 25-hydroxylation vitamin D 324-hydroxylase cDNA. What diluted 1micro of tail DNA preparation objects I with sterilized water 50 times was used for the substrate, first, for 3 minutes, -> with a minute [1 minute] of -> with a seconds [30 seconds] of 94 degrees C 65 degrees C 72 degree-C cycle of 1 minute was repeated 25 times continuously, electrophoresis of the reactant was carried out through agarose GTG (FMC biotechnology product company make) gel 1.0%, and 94 degrees C of rats as which the DNA band of the magnitude of 765bp is regarded were sorted out once. As a result of analyzing the offspring rat of a total of 137 individuals, the PCR positivity individual was one individual. DNA for analysis extracted DNA from about 1cm fragment of a tail by the approach indicated by Hogan and others (1986, MANIPIYU rating THE mouse embryo (cold spring harbor laboratory)). It washed once in 70% ethanol, and the obtained nucleic-acid pellet was dried, and 10mM tris (pH 8.0) of 200microl and 1mM EDTA were made to re-suspend. From the above result, it was checked that a transgenics rat is one individual of individual number R01163-1. The result of injection and a gene analysis is as in a table 2.

[A table 2]

ラットpOsteocalcin-VitaminD3-24hydroxylaseの注入による遺伝子転移ラットの作出

遺伝子	実験番号	卵数		ラット数		
		注入卵数	移植胚数	産仔数	PCR検定個体数	P
pOsteocalcin-VitaminD3-24hydroxylase	43	248	86	19	19	
	44	30	20	8	8	
	45	64	34	6	6	
	46	286	49	24	24	
	47	147	90	35	35	
	48	261	102	45	45	
	計		381	137	137	

[0032] 3) As a result of analyzing two F1 individuals by which the formulation transgenics of a 25-hydroxylation vitamin D 324-hydroxylase transgenics rat was checked by PCR, transgenics was checked by seven animals among [one] 17 animals. 1:1 mating was performed with the Wistar system female rat for the purpose of F1 progeny acquisition of the rat which has the 25-hydroxylation vitamin D 324-hydroxylase gene introduced into the lower stream of a river of MLV-LTR. Consequently, in transgenics rat R9121-7, 12 F1 individuals were acquired and it checked that eight animals were transgenics rats among those as a result of the gene assay by PCR. In the 2nd mating, 14 ** rats were acquired and it checked that six animals were transgenics rats among those. In transgenics rat R11293-5, 14 ** rats were acquired and six animals were transgenics rats among those as a result of the gene assay by PCR. Eleven ** rats were acquired in transgenics rat R12123-6, and four animals were transgenics rats among those as a result of the gene assay by PCR. In the 2nd mating, 11 ** rats were acquired and three animals were transgenics rats among those. The ** rat was acquired in transgenics rat R10175-9 on 12-animal February 12, 1995, and one animal was a transgenics rat among those as a result of the gene assay by PCR. Five ** rats were acquired in transgenics rat R01163-1, and one animal was a transgenics rat among those as a result of the gene assay by PCR. The transgenics rat was not obtained although 12 ** rats were acquired in transgenics rat R02205-1. F1 transgenics rat was obtained by all the transgenics rats except R02205-1 the above result. Abnormality individual for which weight has 39g of elongated incisors by 5 weeks old in the obtained a large number copy transgenics rat (R11293-9) Although checked, it was killed in accident in 5 weeks old. Transgenics rat R12121-7 (female) performed mating twice with one Wistar system male rat, and it acquired 12 ** rats by the eye from one. As a result of performing gene assay by PCR similarly, five animals were transgenics rats among those. Four ** rats were acquired by the eye from two. As a result of PCR's performing gene assay like the above, two animals were transgenics rats among those. Furthermore by R12121-7-2

(female) of this eye from two, and the sib mating of R12121-7-4 (male), 15 ** rats were acquired. PCR performed gene assay and Southern analysis was performed like the above using the DNA ingredient with which transgenics was accepted. Consequently, as compared with the band of F1 obtained individual, the deep thing of a band was judged as a homozygote, and the thing of the band of this level concentration was judged as heterozygote. Furthermore homozygotes were crossed, gene assay was similarly performed by PCR about the after that cost rat, it checked that the individual which a gene is not identified did not exist and it was eventually judged to be a homozygote. Consequently, it became clear that they are five homozygotes, seven heterozygotes, and three non-transgenics rats. the homozygote obtained as mentioned above -- R -- 12121-7-2-1 (male) and R -- 12121-7-2-3 (female) and R -- 12121-7-2-5 (female) and R -- 12121-7-2-11 (female) and R -- it became clear that it is 12121-7-2-12 (male). The schematic diagram is shown in drawing 5 .

[0033] The description 1 of an example 525-hydroxylation vitamin D 324-hydroxylase transgenics rat The quantum of each fraction of the vitamin D3 of the quantum transgenics rat of the vitamin-D3 metabolite in blood of a transgenics rat was performed, and production of a vitamin D deficiency rat was tried in order to investigate this gene expression extent. First, using the transgenics rat and brood rat immediately after 3-weeks old ablactation, vitamin D is removed, respectively and they are calcium 0.5% and P0.6%. At the animal room which carried out optical electric shielding, it bred for one week by acrylic plate manufacturing by AIN-93G corrected purified diet (product made from oriental yeast) and deionized water. Next, vitamin D is removed and they are calcium 0.03% and P 0.15%. It bred by the environmental condition same for four weeks by AIN-93G corrected purified diet (product made from oriental yeast) and deionized water, 2ml collected blood in vitamin D quanta on the 8-weeks old final day, and the blood serum was prepared with the conventional method. The quantum was performed according to the approach indicated by compendiums, such as work (1989, a vitamin handbook (3), (Kagaku-Dojin)) edited by the Vitamin Society of Japan. Measurement of body weight of the each object under vitamin D deficiency low calcium diet breeding was performed weekly. Consequently, the weight of 135 to 167 g and a **** transgenics rat of the weight of the R9121-7F1 female transgenics rat of eight-week ** in vitamin D deficiency low calcium diet breeding was 139 to 157 g. On the other hand, in normal diet breeding, the weight of 206 to 224 g and a **** transgenics rat of the weight of the R9121-7F1 female transgenics rat of eight-week ** was 196g. In vitamin D deficiency low calcium diet breeding, the difference with the amount of 1alpha in a blood serum, 25-hydroxylation vitamin D3, 24, 25-hydroxylation vitamin D3, and 25-hydroxylation vitamin D3 clear between a transgenics rat and a

non-transgenics rat was not seen for the quantum result of each fraction of vitamin D3, and the effectiveness by vitamin D deficiency low calcium diet breeding was not seen. On the other hand, it sets to normal diet breeding and, for 24 and the amount of 25-hydroxylation vitamin D3, three examples are 10.0 ng(s)/ml among four transgenics rats. A non-transgenics rat is seven to 9 ng/ml to having been the above value. It was a value. The amount of 25-hydroxylation vitamin D3 is in the range whose examination individual is 19 to 13 ng/ml, and the difference was not seen. 1alpha and the amount of 25-hydroxylation vitamin D3 -- a non-transgenics rat -- 130 pg(s)/ml and 89 pg/ml it is -- it was 54 to 11 ng/ml in the transgenics rat (table 3 reference).
[A table 3]

VitaminD欠乏飼育による遺伝子転移ラットのVitamin D3各画分の定量

飼育条件		25(OH) Vitamin D3	24,25(OH) Vitamin D3	1α,25(OH) Vitamin D3
VitaminD欠乏飼育				
No.1	遺伝子転移♀	<3ng/ml	1.1ng/ml	30pg/ml
2	遺伝子転移♀	<2	<0.7	65
11	遺伝子転移♀	<3	<0.8	95
3	非遺伝子転移♀	<3	<0.9	81
4	非遺伝子転移♀	<3	<0.8	77
6	非遺伝子転移♀	<3	<0.9	75
正常飼育				
No.7	遺伝子転移♀	19	10.9	37
12	遺伝子転移♀	14	8.3	39
No.13	非遺伝子転移♀	17	7.7	130
14	非遺伝子転移♀	17	8.7	89
R9121-7	遺伝子転移♂	13	11.1	11
R10195-9	遺伝子転移♂	15	10.6	54

[0034] 2) The quantum of the vitamin D metabolite in [of five females (F0 individual, 20 to 40 week **) of quantum 25-hydroxylation vitamin D 324-hydroxylase transgenics rat R02205-1 of the vitamin-D3 metabolite in blood of the transgenics rat which carried out normal breeding, R12123-6, R12121-7, R01163-1, and R11293-5, two R12121-7F1 individual transgenics rats, and two non-transgenics rats] blood was performed. Consequently, the amount of 25-hydroxylation vitamin D3 of a transgenics rat was 28 to 10 ng/ml. As for 24 and the amount of 25-hydroxylation vitamin D3, transgenics rat R12123-6, R01163-1, and R11293-5 were values with few other two individuals than it to having been a 10.0 ng(s)/ml [more than] value. Two

females of R12121-7F1 individual were values with few male 2 individuals than it to having been a 10.0 ng(s)/ml [more than] value. 1alpha and the amount of 25-hydroxylation vitamin D3 -- the transgenics rat R -- any of 02205-1, R12121-7, and F1 non-transgenics individual R12121-7-3 male -- although -- it was 100 or more pg/m and what has 24 [high] and the high amount of 25-hydroxylation vitamin D3 had few 1alpha and amounts of 25-hydroxylation vitamin D3. Each amount of vitamin-D3 metabolite in blood had the large difference between transgenics rat individuals (table 4 reference).

[A table 4]

遺伝子転移ラットのVitamin D3各画分の定量

個体番号	25(OH) Vitamin D3	24, 25(OH) Vitamin D3	1α, 25(OH) Vitamin D3
飼育条件(正常飼育)	ng/ml	ng/ml	pg/ml
① R12121-7 -1 F1♀	21	13.9	8
② R12121-7 -2 F1遺伝子転移♀	28	12.0	7
③ R12121-7 -3 F1 ♂	21	7.7	110
④ R12121-7 -4 F1遺伝子転移♂	10	3.9	82
⑤ R11293-5 Fo遺伝子転移♀	17	12.2	25
⑥ R12121-7 Fo遺伝子転移♀	20	4.7	125
⑦ R12123-6 Fo遺伝子転移♀	16	11.0	52
⑧ R02205-1 Fo遺伝子転移♀	25	6.5	195
⑨ R01163-1 Fo遺伝子転移 ♀	18	13.2	13

[0035] 3) The female individual (individual number 12121-7-3) as which paralysis of lean figure and the limbs, an expansion-and-contraction failure, and a remarkable gait abnormality are regarded after birth as compared with other transgenics rats and brood rats in the transgenics rat of F1 of No.12121-7 in which the abnormality individual in a transgenics rat carried out acquisition breeding was acquired. As a result of the measurement of body weight in 15 ages in day, it was 12g, and 19 to 21 g and a brood female rat were 17 to 23 g, and other female transgenics rats were remarkable individuals with little weight (refer to drawing 6). The morphologic abnormality opinion [in / as compared with a brood rat / in this individual / a bone and a gear tooth] was not acquired. This individual died of 17 ages in day.

[0036] 4) Measurement of body weight of the heterozygote of weight change transgenics rat R12121 -seven-line F2 individual of a 25-hydroxylation vitamin D 324-hydroxylase transgenics rat and a homozygote was performed to 18-week **. Consequently, also in a homozygote and heterozygote, weight

showed the inclination which increases according to ****. The difference in male and female was notably seen after six-week **, respectively, and the weight of 18-week ** was 250 to 400 g for 450 to 600 g, and a female in 300 to 350 g, and a heterozygote male by 450 to 500 g, and the female at the homozygote male. Abnormalities were not accepted in which investigated individual (refer to drawing 7 - drawing 8).

[0037] 5) The twenty-four-hours urine of a transgenics rat was extracted with time using the urine test cage for rats in order to measure the amount of albumin in urine analysis urine of a 25-hydroxylation vitamin D 324-hydroxylase transgenics rat, and the amount of creatinines in urine. According to the conventional method, colorimetry was carried out to measurement of the amount of albumin in urine using a commercial kit, A/G, and B Test Wako (Wako Pure Chem, Inc. make). According to the conventional method, colorimetry was carried out to measurement of the amount of creatinines in urine using a commercial kit and creatinine HA Test Wako HA 7050 (Wako Pure Chem, Inc. make). Furthermore, the amount of albumin in urine / creatinine quantitative ratio in urine was computed from the measured value of an each object. Consequently, R12123-6 female showed the day in 161mg /by 129mg /and 44-week ** the day, and it showed 214mg /, and a day and a high price by 47-week ** by 41-week ** in the transgenics rat F0 generation, and whenever the amount of albumin in urine followed **** further, it increased. Other transgenics rats showed the 7.5 to 37 mg/day value, and the big difference by **** in the individual inside of the body was not accepted. Although, as for the amount of creatinines in urine, the transgenics rat showed the 9.3 to 24.7 mg/day value and the value changed with individuals, the difference by **** in the individual inside of the body was not accepted. R12123-6 female showed 21.7 and a high price by 13.6 or 44-week ** by 41-week ** at 16.4 or 47-week **, and whenever the amount of albumin in urine / creatinine quantitative ratio in urine of an each object followed **** further, it increased. Other transgenics rats showed the value of 0.31-2.47 (table 5 reference).

[A table 5]

遺伝子転移ラットF0世代の尿解析

個体No.	性	世代	週令	Albumin (mg/day)	Creatinine (mg/day)	Alb/Crea
R9121-7	♂	Fo	52	26.1	16.5	1.58
			55	29.1	16.4	1.77
			58	37.0	15.0	2.47
R10175-9	♂	Fo	48	26.1	24.7	1.06
			51	28.9	23.8	1.21
			54	7.51	24.3	0.31
R11293-5	♀	Fo	44	11.0	10.2	1.08
			47	8.0	11.9	0.67
			50	14.8	11.3	1.31
R12121-7	♀	Fo	41	6.50	7.60	0.85
			44	4.80	10.6	0.45
			47	7.05	9.33	1.31
R12123-6	♀	Fo	41	129	9.50	13.6
			44	161	9.80	16.4
			47	214	9.86	21.7
R01163-1	♀	Fo	36	10.7	10.0	1.07
			39	8.70	10.5	0.83
			42	8.10	10.6	0.76
R02205-1	♀	Fo	32	8.30	11.6	0.72
			35	10.3	11.1	0.93
			38	16.8	13.1	1.28

The amount of albumin in urine is set to transgenics rat F1 generation. R12121-7-4 male by 26-week ** 372mg/day 29-week ** showed the day in 352mg /, 31-week ** showed 311mg /, and a day and a high price, other transgenics rat F one-generation R12123-6-3 males and R12123-6-9 male showed the 19 to 28.6 mg/day value, and R12123-6-5 female showed the value of about 11mg/day. The amount of creatinines in urine showed the 7.8 to 26.2 mg/day value by the transgenics rat F1 generation. As for the amount of albumin in urine / creatinine quantitative ratio in urine of an each object, R12121-7-4 male showed 24.5 and a high price by 26.6 or 29-week ** by 26-week ** at 21.5 or 31-week **, and other transgenics rat F1 generations showed the value of 0.45-2.1 (table 6 reference).
[A table 6]

遺伝子転移ラットF世代の尿解析

個体No.	性	世代	週令	Albumin (mg/day)	Creatinine (mg/day)	Alb/Crea
R12121-7-4	♂	F1	26	372	14.0	26.6
			29	352	16.4	21.5
			32	311	12.7	24.5
R12123-6-3	♂	F1	23	26.5	15.7	1.69
			26	22.8	16.8	1.36
			29	20.5	21.6	0.95
R12123-6-5	♀	F1	23	11.3	8.00	1.41
			26	11.6	7.84	1.48
			29	11.9	26.2	0.45
R12123-6-9	♂	F1	23	28.6	13.6	2.10
			26	19.3	10.8	1.79
			29	27.1	14.4	1.88

Although, as for the amount of albumin in urine, transgenics rat F two-generation homozygote R12121-7 -12 male showed the value of 30mg/day or more the day from 11-week **, as for 110mg [/] or more and R12121-7-1 male, as for other homozygote female 3 individuals, the four to 10 mg/day value was shown. Although, as for heterozygote R12121-7 -13 male, 80mg [/] or more and R12121-7 -10 female showed the value after 20mg [/] forward [day] the day from 11-week **, as for other heterozygote male 2 individuals, female 3 individual showed the five to 15 mg/day value ten to 19 mg/the day. As for the male 5 individual average, the contrast rat showed the value of 7.7mg/day the day, as for 12.3mg /and a female 5 individual average. As for the amount of creatinines in urine, in male 2 individual, in the homozygote, female 3 individual showed the seven to 11 mg/day value ten to 15 mg/the day. In male 3 individual, in heterozygote, female 4 individual showed the five to 10 mg/day value eight to 16 mg/the day. As for the contrast rat, the male 5 individual average showed the value 13.5mg/[a day and] and whose female 5 individual average are 9.2mg/day. Although the amount of albumin in urine / creatinine quantitative ratio in urine of an each object were [8.0 or more and R12121-7-1 male of homozygote R12121-7 -12 male] 2.8 or more, other homozygote female 3 individuals were 0.5-1.3. In heterozygote, 9.0 or more and R12121-7 -10 female of R12121-7 -13 male were 2.4 or more. 0.9-1.8 and female 3 individual of other heterozygote male 2 individuals were 0.8-2.4. The contrast rat was [0.9 and the female 5 individual average of the male 5 individual average] 0.8 (table 7 reference).

[A table 7]

遺伝子転移ラットF2世代の尿解析

系統；R12121-7由来F2個体(ヘテロおよびホモ接合体)

アルブミン量

個体No.	性	Albumin (mg/day)						
		11w	14w	17w	21w	24w	27w	30w
ヘテロ接合体								
7-4	♀	5.9	6.6	9.4	3.6	8.7	16.2	9.3
7-6	♂	16.3	18.3	10.8	-	-	-	-
7-7	♀	14.9	10.1	7.5	8.6	13.5	28.9	23.9
7-8	♂	18.1	15.7	14.1	19.7	16.4	23.5	18.0
7-10	♀	23.1	17.0	28.9	48.5	60.5	54.8	52.4
7-13	♂	88.0	84.5	115.8	198.0	210.0	341.2	303.7
7-15	♀	11.3	14.5	11.2	21.1	24.6	57.2	42.1
ホモ接合体								
7-1	♂	32.0	39.4	77.3	124.5	244.0	370.0	-
7-3	♀	5.4	9.1	6.4	17.5	11.8	18.6	12.6
7-5	♀	9.1	9.9	4.9	8.8	7.6	11.5	11.1
7-11	♀	9.0	9.4	4.6	7.1	10.5	21.1	10.9
7-12	♂	118.0	120.0	169.0	277.3	343.0	441.3	439.4

遺伝子転移ラットF2世代の尿解析

系統；R12121-7由来F2個体(ヘテロおよびホモ接合体)

アルブミン量

個体No.	性	Albumin (mg/day)						
		11w	14w	17w	21w	24w	27w	30w
対照								
1	♂	14.6	13.0	31.4	9.3	36.6	22.4	
2	♂	11.8	21.7	13.7	11.6	22.1	15.6	
3	♂	13.9	13.4	11.2	10.3	16.9	6.8	
4	♂	7.2	9.3	24.1	13.8	17.0	8.1	
5	♂	14.1	12.6	20.4	14.6	29.5	3.9	
Mean±S.D		12.3±3.1	14.0±4.6	20.2±8.1	11.9±2.3	24.4±8.5	11.4±7.5	
対照								
1	♀	5.6	13.4	7.7	9.4	16.2	6.1	
2	♀	10.6	7.5	9.1	12.3	5.5	29.1	
3	♀	9.7	10.1	4.0	8.0	22.7	14.6	
4	♀	5.6	4.2	13.9	4.5	17.7	13.1	
5	♀	7.1	6.9	11.2	7.1	19.6	49.6	
Mean±S.D		7.7±2.3	8.4±3.5	9.2±3.7	8.3±2.9	17.8±8.1	22.5±17.3	

遺伝子転移ラットF2世代の尿解析

系統；R12121-7由来F2個体(ヘテロおよびホモ接合体)

クレアチニン量

個体No.	性	Creatinine (mg/day)						
		11w	14w	17w	21w	24w	27w	30w
ヘテロ接合体								
7-4	♀	5.9	8.0	7.7	8.6	13.4	8.8	7.3
7-6	♂	11.4	16.0	11.2	-	-	-	-
7-7	♀	6.2	8.5	8.3	9.9	8.6	8.4	9.0
7-8	♂	10.4	13.9	11.8	16.9	16.4	17.2	14.7
7-10	♀	5.9	7.0	9.4	11.0	9.0	9.4	8.8
7-13	♂	8.3	9.3	12.1	12.4	9.5	16.7	15.6
7-15	♀	5.9	7.2	7.4	12.4	7.7	6.9	6.2
ホモ接合体								
7-1	♂	10.7	13.8	17.8	14.2	13.4	13.2	-
7-3	♀	8.9	10.5	16.7	10.9	11.2	10.4	9.3
7-5	♀	9.0	10.5	20.3	10.7	9.8	9.9	9.5
7-11	♀	7.2	8.8	16.5	12.1	11.4	9.8	7.9
7-12	♂	12.9	14.4	15.1	16.3	13.9	17.4	15.6

遺伝子転移ラットF2世代の尿解析

系統；R12121-7由来F2個体(ヘテロおよびホモ接合体)

クレアチニン量

個体No.	性	Creatinine (mg/day)						
		11w	14w	17w	21w	24w	27w	30w
対照								
1	♂	13.9	18.0	17.8	21.0	18.6	15.4	
2	♂	12.3	21.2	16.7	16.7	16.7	12.4	
3	♂	14.7	43.4	20.3	18.9	20.7	9.0	
4	♂	11.5	14.2	16.5	16.1	15.5	19.0	
5	♂	15.2	16.8	15.1	19.1	13.7	5.2	
	Mean±S.D	13.5±1.6		17.3±1.9		17.0±2.7		
			22.7±11.8		18.4±2.0		12.2±5.4	
対照								
1	♀	10.4	10.2	11.9	11.5	10.0	11.4	
2	♀	10.1	11.5	4.4	11.5	10.3	19.5	
3	♀	10.9	11.3	8.8	11.4	11.5	27.2	
4	♀	7.1	5.5	11.2	9.5	9.1	8.5	
5	♀	7.5	10.7	10.4	9.9	10.9	35.4	
	Mean±S.D	9.2±1.8		9.3±3.0		10.4±1.3		
			9.8±2.5		10.8±1.0		20.4±11.1	

遺伝子転移ラットF2世代の尿解析

系統；R12121-7由来F2個体(ヘテロおよびホモ接合体)

アルブミン量/クレアチニン量

個体No.	性	Albumin / Creatinine						
		11w	14w	17w	21w	24w	27w	30w
ヘテロ接合体								
7-4	♀	1.00	0.83	0.80	0.42	0.65	1.84	1.27
7-6	♂	1.43	1.14	0.96	-	-	-	
7-7	♀	2.40	1.19	0.90	0.87	1.57	3.44	2.66
7-8	♂	1.74	1.13	1.19	1.17	1.00	1.37	1.22
7-10	♀	3.92	2.45	3.07	4.41	6.72	5.83	5.95
7-13	♂	10.6	9.11	9.57	16.0	22.1	20.4	19.5
7-15	♀	1.42	2.02	1.52	3.35	3.19	8.3	6.79
ホモ接合体								
7-1	♂	2.99	2.86	5.44	8.77	18.2	28.0	
7-3	♀	0.61	0.87	0.80	1.61	1.05	1.79	1.35
7-5	♀	1.01	0.94	1.22	0.82	0.78	1.16	1.17
7-11	♀	1.25	1.06	0.46	0.59	0.92	2.15	1.38
7-12	♂	9.15	8.36	11.4	17.0	24.7	25.4	28.2

遺伝子転移ラットF2世代の尿解析

系統；R12121-7由来F2個体(ヘテロおよびホモ接合体)

アルブミン量/クレアチニン量

個体No.	性	Albumin / Creatinine						
		11w	14w	17w	21w	24w	27w	30w
対照								
1	♂	1.05	0.72	1.76	0.44	1.97	1.45	
2	♂	0.96	1.02	0.82	0.69	1.32	1.26	
3	♂	0.95	0.31	0.55	0.54	0.82	0.76	
4	♂	0.63	0.65	1.46	0.86	1.10	0.43	
5	♂	0.93	0.75	1.35	0.76	2.15	0.75	
Mean±S.D		0.90±0.16	0.69±0.25	1.19±0.49	0.66±0.17	1.47±0.6	0.93±0.4	
対照								
1	♀	0.54	1.31	0.65	0.82	1.62	0.53	
2	♀	1.05	0.66	2.06	1.07	0.53	1.49	
3	♀	0.89	0.89	0.45	0.70	1.97	0.54	
4	♀	0.79	0.71	1.24	0.47	1.95	1.54	
5	♀	0.95	0.65	1.08	0.72	1.80	1.80	
Mean±S.D		0.84±0.19	0.84±0.28	1.10±0.63	0.76±0.22	1.75±0.8	1.10±0.5	

[0038] 6) Blood collecting of a transgenics rat was performed according to the conventional method in order to measure the biochemical analysis blood medium maturing chemistry marker of the blood of a 25-hydroxylation vitamin D 324-hydroxylase transgenics rat. Measurement of the total cholesterol

(TCHO) in blood performed colorimetry using the commercial kit (the Fuji film incorporated company make). Measurement of the amount of ****** triglycerides (TG) performed colorimetry using the commercial kit (the Fuji film incorporated company make). Measurement of the amount (GLU) of ****** glucoses performed colorimetry using the commercial kit (the Fuji film incorporated company make). Measurement of the amount of calcium in blood (calcium) performed colorimetry using commercial kit calcium E-Test Wako (Wako Pure Chem, Inc. make). Measurement of the amount of Lynn in blood (P) performed colorimetry using commercial kit phospholipid C-Test Wako (Wako Pure Chem, Inc. make). Colorimetry was carried out to measurement of the amount (CREA) of creatinines in blood using the commercial kit (the Fuji film incorporated company make). Furthermore, the creatinine clearance (a part for /[ml] /100g weight) (Ccr) of an each object was computed by the amount of creatinines / weight x100/1440 among the amount of amount of creatinines in urine x twenty-four-hours urine / blood from the measured value of the amount of creatinines in blood of an each object, and the amount of creatinines in urine. Consequently, in the transgenics rat F0 generation, as for the total cholesterol in blood, R12123-6 female showed the high price of 200 or more mg/dl. Other transgenics rats showed the value of 60-150 mg/dl. In R12123-6, the amount of triglycerides in blood was 2265 mg/dl, and was a high price also in R11293-5 and R02205-1. The difference in a transgenics rat with the big amount of blood glucose was not seen. The difference in a transgenics rat with the big amount of calcium in blood was not seen. The amount of Lynn in blood was the high price of 752 mg/dl in R12123-6. The creatinine clearance was the low value of under /100g weight by 0.2ml/in R09121-7. In the transgenics rat F1 generation, as for the total cholesterol in blood, R12121-7-4 female showed 228 mg/dl and a high price. Other transgenics rats showed the value of 64-120 mg/dl. The amount of triglycerides in blood was the high price of 331 mg/dl in R12121-7-4. The difference in a transgenics rat with the big amount of blood glucose was not seen. The difference in a transgenics rat with the big amount of calcium in blood was not seen. The amount of Lynn in blood was the high price of 375 mg/dl in R12121-7-4. The difference about the calculated individual with the big creatinine clearance was not seen. (Table 8 reference) .

[A table 8]

MLV-LTR-VitaminD3-24Hydroxylase遺伝子転移ラットF1,F2世代の血液解析

系統No.	血清 TCHO (mg/dl)	TG (mg/dl)	GLU (mg/dl)	Ca (mg/dl)	P (mg/dl)	CREA (mg/dl)	Ccr (ml/min/100g.bwt)
F0							
R09121-7	124	107	116	9.4	165	0.7	0.173
R10175-9	150	286	136	9.7	272	0.6	0.411
R11293-5	82	640	108	9.3	258	0.6	0.282
R12121-7	78	271	114	8.9	214	0.6	0.261
R12123-6	233	2265	114	8.9	752	0.6	0.254
R01163-1	60	191	120	10.0	164	0.7	0.231
R02205-1	68	394	111	9.5	214	0.7	0.234
F1							
R12121-7-4	228	331	117	9.7	375	1.2	
R12123-6-3	120	183	158	10.0	221	0.5	0.292
R12123-6-5	64	200	146	9.6	189	0.7	0.294
R12123-6-9	90	205	123	9.5	210	0.6	0.243

As for the total cholesterol in blood, transgenics rat F2 generation R12121-7-12 and -13 male showed the high price before and behind 200 mg/dl by 24-week ** to average 71 mg/dl of a contrast male rat, and average 84 mg/dl of a contrast female rat. The amount of triglycerides in blood was the high price of 400 or more mg/dl in R12121-7-12 and -13 to average 95.6 mg/dl of a contrast male rat, and average 144 mg/dl of a contrast female rat. The transgenics rat of the amount of blood glucose is 86-131 mg/dl to average 122 mg/dl of a contrast male rat, and average 124 mg/dl of a contrast female rat, and the big difference was not seen. The transgenics rat of the amount of calcium in blood is 8.8-10.1 mg/dl to average 9.2 mg/dl of a contrast male rat, and average 9.7 mg/dl of a contrast female rat, and the big difference was not seen. The amount of Lynn in blood was the high price of 300 or more mg/dl in R12121-7-12 and -13 to average 126 mg/dl of a contrast male rat, and average 174 mg/dl of a contrast female rat. As for the creatinine clearance, R12121-7-4, -10, -1, -11, -12, and -13 showed less than 0.3 value to /100g weight by average /100g weight and average [of a contrast female rat / of 0.366ml]/of 0.376ml of a contrast male rat (table 9 reference). [part / for /]

[A table 9]

MLV-LTR-VitaminD3-24Hydroxylase遺伝子転移ラットの血液解析

系統；R12121-7由来F2、1産目個体（24週齢）

個体No.	血清 TCHO (mg/dl)	TG (mg/dl)	GLU (mg/dl)	Ca (mg/dl)	P (mg/dl)	CREA (mg/dl)	Ccr (ml/min/100g.bwt)
ヘテロ接合体							
7-4	69	221	124	9.1	211	0.7	0.252
7-7	79	189	111	10.1	203	0.5	0.382
7-8	81	175	114	8.8	187	0.6	0.356
7-10	88	105	119	8.9	185	0.6	0.212
7-13	201	400	100	9.4	316	0.7	0.208
7-15	95	248	115	9.4	160	0.6	0.308
ホモ接合体							
7-1	147	183	131	9.4	249	0.6	0.277
7-3	72	371	122	9.5	222	0.6	0.354
7-5	73	390	86	9.5	230	0.5	0.360
7-11	56	162	129	9.2	136	0.8	0.212
7-12	198	439	92	9.7	364	0.7	0.282
対照							
1(♂)	82	98	114	9.4	158	0.7	0.330
2	89	108	120	9.7	132	0.5	0.418
3	63	98	134	9.3	115	0.6	0.423
4	59	86	121	8.9	115	0.7	0.328
5	61	88	122	8.9	110	0.7	0.383
Mean±S.D	71±13.7	95.6±8.9	122±7.3	9.2±0.3	126±20	0.64±0.09	0.376±0.05
6(♀)	82	198	105	10.0	172	0.8	0.412
7	71	110	118	9.6	206	0.8	0.358
8	90	169	138	9.5	165	0.6	0.364
9	73	94	125	9.5	164	0.9	0.386
10	104	151	136	10.0	162	0.7	0.308
Mean±S.D	84±13.5	144±42.6	124±13.6	9.7±0.3	174±18	0.76±0.11	0.366±0.04

By the same 27-week ** of an individual, as for R12121-7-12 and -13, the total cholesterol in blood showed the high price of 200 or more mg/dl to average 62.4 mg/dl of a contrast male rat, and average 70.6 mg/dl of a contrast female rat. As for the amount of triglycerides in blood, R12121-7-7, -1, -3, -12, and -13 were 300 or more mg/dl to average 113 mg/dl of a contrast male rat, and average 166 mg/dl of a contrast female rat. The transgenics rat of the amount of blood glucose is 113-156 mg/dl to average 149 mg/dl of a contrast male rat, and average 117 mg/dl of a contrast female rat, and the big difference was not seen. The transgenics rat of the amount of calcium in blood is 8.2-10.2 mg/dl to average 9.1 mg/dl of a contrast male rat, and average 9.5 mg/dl of a contrast female rat, and the big difference was not seen. the amount of Lynn in blood -- average 129 mg/dl of a contrast male rat, and average 172 mg/dl of a contrast female rat -- receiving -- R12121-7- it was the high price of 300 or more mg/dl in 1, 12,

and -13. Although, as for the creatinine clearance, R12121-7-10, -1, -7, -15, -11, -5, and -12 showed less than 0.3 value to /100g weight by average /100g weight and average [of a contrast female rat / of 0.442ml]/of 0.365ml of a contrast male rat and those values changed with **** in the same individual, the individual which shows outlying observation was the almost same individual (table 10 reference). [part / for /]

[A table 10]

MLV-LTR-VitaminD3-24Hydroxylase遺伝子転移ラットの血液解析

系統；R12121-7由来F2、1産目個体（27週齢）

	血清						
個体No.	TCHO (mg/dl)	TG (mg/dl)	GLU (mg/dl)	Ca (mg/dl)	P (mg/dl)	CREA (mg/dl)	Ccr (ml/min/100g-bwt)
ヘテロ接合体							
7-4	80	182	129	9.5	191	0.6	0.315
7-7	74	300	133	10.0	218	0.7	0.254
7-8	78	139	156	9.2	164	0.6	0.358
7-10	86	57	118	8.2	161	1.2	0.154
7-13	206	341	113	9.2	331	0.7	0.362
7-15	87	268	128	9.6	217	0.6	0.259
ホモ接合体							
7-1	184	312	136	10.0	327	0.6	0.264
7-3	75	369	136	9.9	226	0.6	0.326
7-5	87	211	133	9.4	203	0.7	0.256
7-11	63	63	134	8.6	136	1.4	0.135
7-12	233	498	118	10.2	402	0.8	0.295
対照							
1(♂)	73	144	138	9.4	156	0.6	0.317
2	79	130	157	9.7	146	0.5	0.310
3	58	117	150	9.2	124	0.6	0.384
4	50	89	149	8.8	108	0.7	0.404
5	52	87	149	8.6	111	0.7	0.410
		113±2.5		9.1±0.3		0.62±0.09	
Mean±S.D	62.4±12.9		149±6.8		129±21.3		0.365±0.05
6(♀)	78	112	117	8.7	180	0.8	0.392
7	52	212	109	9.4	161	0.8	0.505
8	84	178	129	10.1	168	0.6	0.520
9	62	164	102	9.7	154	0.7	0.368
10	77	164	127	9.7	195	0.8	0.424
		166±36		9.5±0.5		0.74±0.1	
Mean±S.D	70.6±13.2		117±11.5		172±16.2		0.442±0.07

7) pathology histological analysis transgenics rat F2 generation [of a 25-hydroxylation vitamin D 324-hydroxylase transgenics rat] R -- after slaughtering 12121-7-1 (a homozygote, 28 week ** of males), and a contrast rat, from the each object, a brain, the heart, liver, lungs, the kidney, the spleen, the testis, and the femur were started with the conventional method, and it fixed by immersing each in a formalin solution 10%. According to the conventional method, the intercept was produced with paraffin embedding,

then a microtome, the hematoxylin eosin stain was performed and the sample was produced. a femur -- 10% formic acid -- using it -- the deliming processing for three days -- a conventional method -- following -- carrying out -- a sagittal plane -- as -- a broth and other organs -- the same -- a sample -- having produced . The produced sample observed under the optical microscope. Consequently, in R12121-7-1 and a contrast rat, change was not seen for the cerebrum. In R12121-7-1 and a contrast rat, change was not seen for the cerebellum. As for liver, the cellular infiltration of a Glisson's capsule was observed in the contrast rat. On the other hand, in R12121-7-1, the cellular infiltration of a Glisson's capsule, the vacuolation of hepatocyte, and the nature change of a disseminated clear cell were observed. In the contrast rat, change was not seen for the kidney. On the other hand, in R12121-7-1, an escape and small round cell infiltration of good basification and the urinary casts of the glomerulosclerosis, a nephropathy, ureter simple hypermorphosis, and a renal tubule epithelium, and a renal tubule were observed. In the contrast rat, change was not seen for lungs. On the other hand, in R12121-7-1, the thickening of the mineralization of the thickening of the media of the smallness of lungs and an inside artery and adventitia, the alveolus histiocytosis, osseous metaplasia, and the pulmonary artery and the alveolar septum of locality was observed. In R12121-7-1 and a contrast rat, change was not seen for the heart. As for the spleen, extramedullary hematopoiesis and pigmentation were observed in the contrast rat. On the other hand, in R12121-7-1, extramedullary hematopoiesis sthenia and artery thickening were observed. In the contrast rat, change was not seen for bone marrow. On the other hand, hematogenous sthenia was observed in R12121-7-1. In R12121-7-1 and a contrast rat, change was not seen for the testis.

8) After slaughtering organ weight transgenics rat F4 generation R12121-7-1 (the homozygote, 18 week ** of males) and the contrast rat of a 25-hydroxylation vitamin D 324-hydroxylase gene transgenic rat, from the each object, the heart, liver, lungs, the kidney, and a spleen were started with the conventional method, and each gravimetry was performed. Consequently, the weight of the heart, liver, lungs, and a spleen was intentionally high as compared with the contrast rat. As for the weight of the kidney, the high inclination was seen as compared with the contrast rat.

9) bone-salt consistency transgenic rat F3 generation [of a 25-hydroxylation vitamin D 324-hydroxylase gene transgenic rat] R -- 12121-7-1 -- and (a homozygote, 16-week **) after slaughtering a contrast rat, from the each object, the femur and the tibia were started and each bone-salt consistency was measured by the duplex energy X-ray absorption measuring method (general -- DXA -- abbreviated to law). Consequently, as for the bone-salt consistency of the tibia juxtaposition edge 1 / 3 section of a sex transgenic

rat, significant lowering was accepted compared with each contrast rat. Furthermore, the inclination for the bone-salt consistency of the femur distal end 1 / 3 section of a male transgenic rat to fall compared with each contrast rat was accepted. 10) According to the conventional method, in guanidine, histotripsy of the RNA for gene expression analysis analysis of a 25-hydroxylation vitamin D 324-hydroxylase transgenics rat was carried out, and it was extracted from a part of the brain of above transgenics rat F2 generation R12121-7-1 (a homozygote, 28 week ** of males), and each contrast rat, the heart, liver, lungs, the kidney, spleen, testis, and femur. The obtained nucleic-acid pellet was washed once in 70% ethanol, and sterilized water was made to re-suspend after desiccation. The RIBASUTORANSU polymerase chain reaction method (generally called RT-PCR method) was performed using the above-mentioned primers 5 and 6. 20micro of RNA preparation objects g was used for the substrate, first, by the reverse transcriptase, 60 degrees C continued for 30 minutes, and 94 degrees C was processed for 2 minutes. After adding Taq polymerase and repeating -> with a minute [1 minute] of 94 degrees C 60 degree-C reaction for 1.5 minutes 40 times, processing for [60 degrees-C] 7 minutes was performed, and electrophoresis was carried out to agarose GTG (FMC biotechnology product company make) gel through the reactant 1.0%. In the contrast rat, a DNA band was not seen in all the analyzed organs. On the other hand, in R12121-7-1, the DNA band of 400bp(s) was accepted in all the analyzed organs (refer to drawing 9). In the brain, the heart, the liver, the lungs, the kidney, the spleen, testis, and femur of transgenics rat F2 generation R12121-7-1 (a homozygote, 28 week ** of males), 25-hydroxylation vitamin D 324-hydroxylase gene expression was seen, and it became clear by contrast that the 25-hydroxylation vitamin D 324-hydroxylase gene expression of internality is also below limit of detection.

[0039]

[Effect of the Invention] The nonhuman mammal into which the 25-hydroxylation vitamin D 324-hydroxylase gene of this invention was introduced A hypercalcemia, a hypocalcemia, hyperparathyroidism, rickets, Bone diseases, such as osteomalacia, osteoporosis, and osteopenia, glomerulonephritis, the glomerulosclerosis, To kidney diseases, such as chronic nephritis and renal failure, and a pan, an articular disease, a lung disease, hyperlipidemia, The symptoms of arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, cancers, or those complication may be shown, and it can use as the symptoms model animal. For example, it is possible to perform break through of these symptoms mechanisms, examination of the prevention / therapy approach of a disease, and screening of a remedy using the rat of this invention. The

transgenic animal of this invention is applicable to the therapy model of the disease resulting from the vitamin-D3 metabolic error which includes the break-through model, the above-mentioned disease, and the above-mentioned disease of a symptoms function as a symptoms model of kidney disease, such as bone diseases, such as osteoporosis and osteomalacia, and a nephritis, and renal failure, screening of the candidate compound in remedy development, and the cell supply used for those in vitro assessment. By this invention, the superfluous manifestation of 25-hydroxylation vitamin D 324-hydroxylase mainly promotes the vitamin-D3 metabolic turnover imbalance of the kidney. The bone disease which minded inactivation or lowering for the function of a gene to adjust activation mold vitamin D3, Kidney disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, It is characterized by authorizing the improvement effect of obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, or cancer. The mechanism whose symptoms kidney disease, a bone disease, an articular disease, a lung disease, hyperlipidemia, arteriosclerosis, a heart disease, diabetes mellitus, obesity, a digestive system disease, an infectious disease, the allergosis, an endocrinologic disease, Alzheimer's disease, cancers, or those complication show was solved. Especially in renal failure, it became clear to be based on a vitamin-D3 metabolic error, especially vitamin D 324-overhydroxylase manifestation. This data is new knowledge. The nonhuman mammal into which the 25-hydroxylation vitamin D 324-hydroxylase gene of this invention was introduced can be used also for the object which solves the target gene controlling mechanism of supply of a 25-hydroxylation vitamin D 324-hydroxylase gene high manifestation cell, and a nucleus interoception object.

[0040]

[Layout Table]

[array number: 1]

die-length [of an array]: -- mold [of 21 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array Synthetic DNA array AGGCTGTGCT GCTAATGTCA A 21 [0041]

[array number: 2]

die-length [of an array]: -- mold [of 21 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array Synthetic DNA array AAGAGTGGGG GTCAGAGTTC G 21 [0042]

[array number: 3]

die-length [of an array]: -- mold [of 24 arrays]: -- number [of

nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array Synthetic DNA array TGAGGACATT ACTGACCGCT CCTT 24 [0043]
[array number: 4]
die-length [of an array]: -- mold [of 24 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array Synthetic DNA array AGTTGCTGTG TGGGACTTGT CTGT 24 [0044]
[array number: 5]
die-length [of an array]: -- mold [of 21 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array Synthetic DNA array CTGTCTTCTT TCAACCTGGA T 21 [0045]
[array number: 6]
die-length [of an array]: -- mold [of 21 arrays]: -- number [of nucleic-acid chains]: -- single strand topology: -- nucleic acid besides class: of a straight chain-like array Synthetic DNA array TTAGAGTTCT GTGGGGCATT C 21 [0046]

[Translation done.]

DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] Construction drawing of plasmid pMLV-LTR-VitaminD324-Hydroxylase.
[Drawing 2] The result of the Southern analysis of the transgenics rat by the RIBOPU lobe of 600bp(s) which carried out the JIKOKISHI genin indicator (each sample: 10microg; alkali blotting).
Rain 1: Marker (lambda/HindIII+phi x174-/HaeIII)
Lane 2: Negative control
Rain 3: R12123-6 (+)
Rain 4: R12121-7 (+)
Rain 5: R10175-9 (?)
Rain 6: R11293-5 (+)
Rain 7: R11293-9 (+)
[Drawing 3] The result of the Southern analysis of the transgenics rat by the probe of the restriction enzyme XbaI-KpnI940bp fragment which carried out the random prime indicator.
Rain 1: Marker (MLV-24-hydroxylase / ClaI)
Rain 2: R9121-7/ClaI
[Drawing 4] Construction drawing of plasmid pOsteocalcin-VitaminD324-hydroxylase.
[Drawing 5] The schematic diagram of transgenics rat R12121-7.
[Drawing 6] The abnormality individual of a MLV-LTR-VitaminD324-hydroxylase transgenics rat (15 ages in day).
[Drawing 7] Weight change of transgenics rat R12121-7F2 individual (homozygote).
[Drawing 8] Weight change of transgenics rat R12121-7F2 individual (heterozygote).
[Drawing 9] The gene expression analysis of transgenics rat F2 generation R12121-7-1 is shown (RT-PCR: electrophoresis).

[Translation done.]